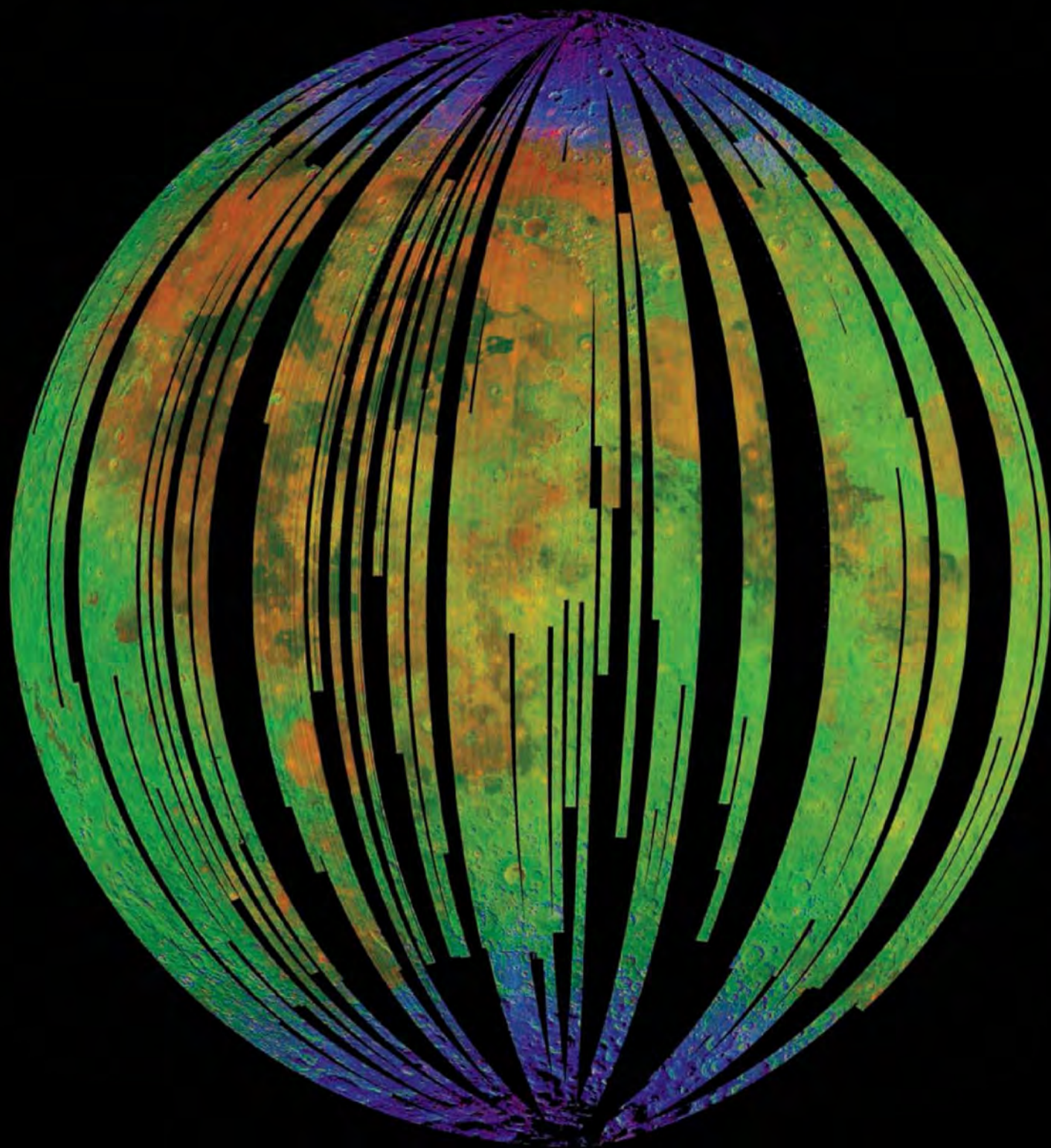


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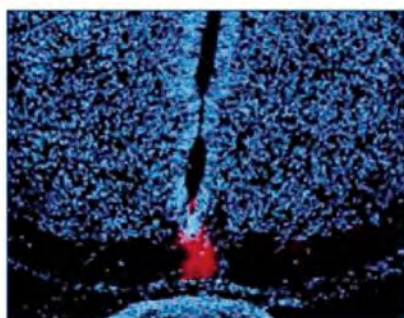
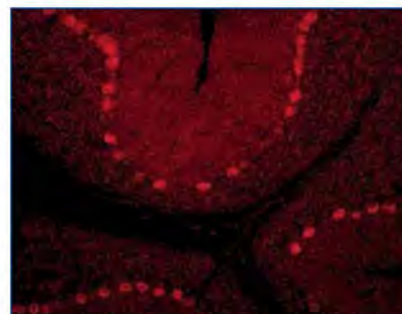
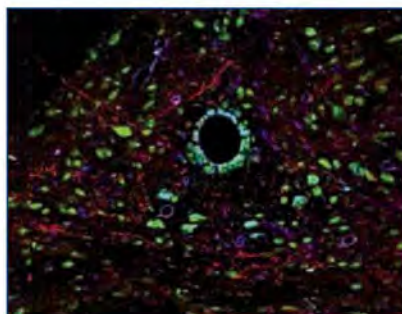
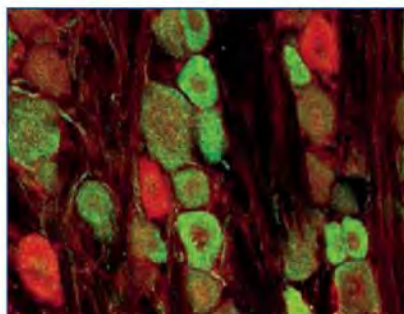
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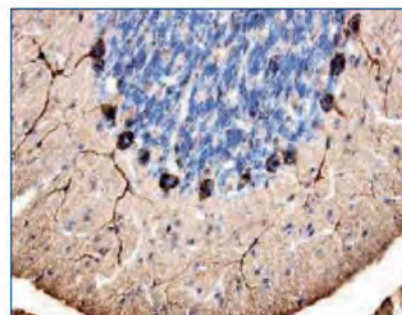
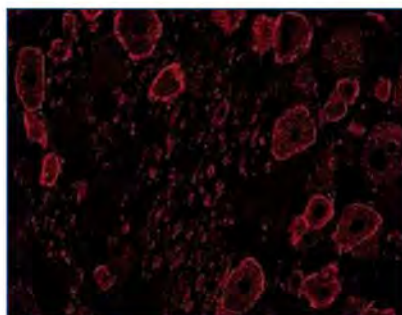
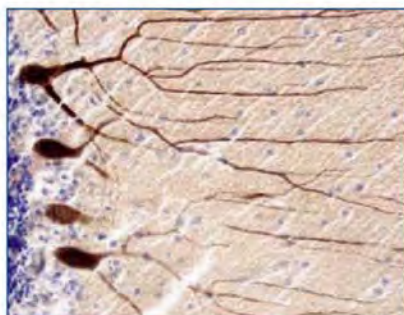
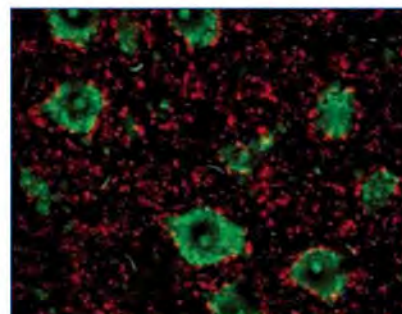
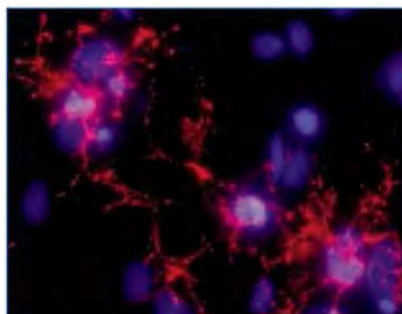
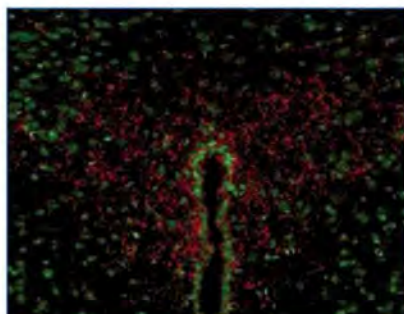
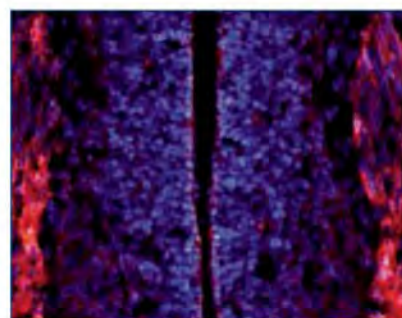
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A three-color mosaic derived from the Moon Mineralogy Mapper (M^3) near-infrared spectrometer. Orange and pink colors illustrate the distribution of iron-bearing minerals. Green represents the surface brightness at 2.4 micrometers. Blue indicates the presence of small amounts of surficial OH and H_2O that are most prominent at these viewing geometries at cooler, higher latitudes. See page 568.

Image: NASA/ISRO/Brown University/R. N. Clark, USGS

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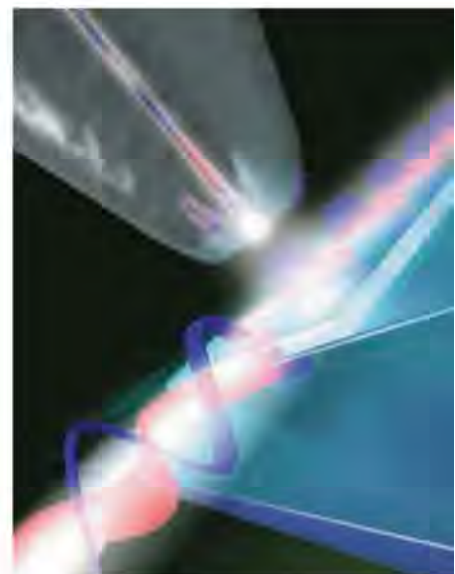
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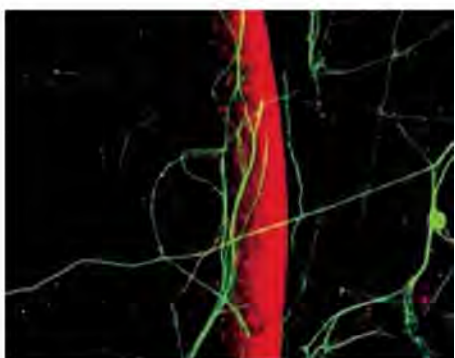
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Structural Mechanism of Abscisc Acid Binding and Signaling by Dimeric PYR1

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The plant hormone responsible for drought tolerance signals by inducing conformational changes in its dimeric protein receptor.
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10.1126/science.1178712

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10.1126/science.1176593

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RESEARCH ARTICLE: Slow Elimination of Multidrug-Resistant Tuberculosis

C. Dye and B. G. Williams

The spread of drug-resistant TB can be reversed through better treatment and diagnosis.

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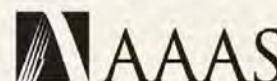
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Croaking Frogs >>

The global amphibian decline has been attributed, among other causes, to an amphibian skin disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis*. However, how this pathogen causes mortality has been unclear. **Voyles *et al.*** (p. 582) show that this superficial skin infection may lead to cardiac failure owing to changes caused by lowered ion transport through the skin and consequent electrolyte reduction in the blood.



Drosophila Body Color

Fly body color is controlled by a variety of genes and alleles. Now **Wittkopp *et al.*** (p. 540) describe how two genes at the ebony and tan genetic loci control body color among two closely related species, *Drosophila americana* and *D. novamexicana*. Variations at the tan locus and linked to the ebony locus also contribute to intraspecific pigmentation changes with geography in *D. americana*. The sequencing of multiple isolates suggests that some strains of *D. americana* carry alleles of tan and ebony that are more closely related to the *D. novamexicana* alleles than they are to other *D. americana* alleles. Thus, the genetic determinants of both inter- and intra-species color variation is due to shared alleles.

Yeast Joins RNAi Club

RNA interference (RNAi) silences gene expression via small interfering (si) RNAs that bind to target sequences. RNAi has been found in almost all eukaryotes examined, with the notable exception of the budding yeast, *Saccharomyces cerevisiae*, one of the most prominent organisms used in the study of molecular biology. Indeed, RNAi has been thought to be missing from all budding yeast. **Drienen *et al.*** (p. 544, published online 10 September; see the Perspective by **Moazed**) now show that several species of budding yeast do possess RNAi, which seems to act primarily on transposable elements and subtelomeric repeats. A noncanonical Dicer protein generates siRNAs that are loaded onto



Ago1 protein in *S. castellii*. Introduction of these two genes into *S. cerevisiae* was sufficient to reconstitute RNAi, where it also acted to dampen the activity of transposons.

Measuring Magnetic Light

Compared to its electric component, the coupling between the magnetic field component of light and matter is usually extremely weak. With no effective way to measure it, we are effectively blind to light's magnetic component. **Buresi *et al.*** (p. 550, published online 1 October; see the Perspective by **Giessen and Vogelgesang**) coupled a metamaterial split-ring resonator to the tip of a scanning probe to measure the magnetic field vector of light at optical frequencies. The ability to measure the magnetic component of light should prove useful for the nanoscale characterization of optical waveguides and other optical devices.

Lunar Water

The Moon has been thought to be primarily anhydrous, although there has been some evidence for accumulated ice in permanently shadowed craters near its poles (see the Perspective by **Lucey**, published online 24 September). By analyzing recent infrared mapping by Chandrayaan-1 and Deep Impact, and reexamining Cassini data obtained during its early flyby of the Moon, **Pieters *et al.*** (p. 568, published online 24 September), **Sunshine *et al.*** (p. 565, published online 24 September), and **Clark *et al.*** (p. 562, published online 24 September) reveal a noticeable absorption signal for H₂O and OH across much of the surface. Some variability in water abundance is seen over the course of the lunar day. The data imply that solar wind is depositing and/or somehow forming water and OH in minerals near the lunar surface, and that this trapped water is dynamic.

Life in Dead Zones

Oxygen minimum zones, or oceanic "dead zones," are expanding. Marine dead zone expansion and intensification results in amplification of biological nitrogen and greenhouse gas production, with implications for marine fisheries' productivity and for climate balance. Little is known about the microbial communities mediating the underlying biogeochemistry of dead zones. **Walsh *et al.*** (p. 578) present metagenomic analyses of a ubiquitous, abundant, and uncultivated bacterium in oceanic dead zones. Similar bacteria, related to chemoautotrophic gill symbionts of deep-sea clams and mussels, play a role in sulfide detoxification in African shelf waters. Reconstruction of the carbon and energy metabolism of this enigmatic lineage revealed a metabolic repertoire mediating carbon sequestration, sulfur-detoxification, and biological nitrogen loss in oxygen-deficient oceanic waters.

Observing Unrevealed Preferences

Ideally, it would be possible to design a system of incentives for the production and allocation of public goods with the following properties: (i) it would be budget-balanced; (ii) people would participate willingly because they would not be made worse off by doing so; (iii) it would be easy for each participant to find his or her optimal strategy; and (iv) the equilibrium solution would yield the optimal production of the public good. Sadly, this set of conditions cannot be satisfied simultaneously because it requires that self-interested individuals reveal voluntarily and truthfully how much they value the public good. **Krajbich *et al.*** (p. 596, published online 10 September) ask whether a neuroimaging mea-

surement can be used to circumvent this reliance on observed behavior by decoding individual valuations. A decoding accuracy of 55% would be sufficient, and, in a laboratory experiment, an optimal provision of public goods was indeed achieved.

Methane Loosely Bound

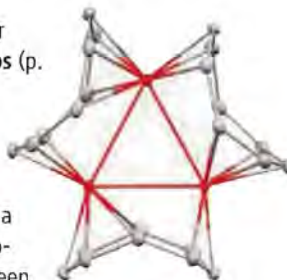
For the most part, molecular bonds involve sharing of electrons between two discrete atoms. In certain cases, however, a third atom can also attract a portion of the electron density without fully cleaving the bond. Such loose complexes between C-H bonds and transition metals are often invoked as short-lived intermediates in metal-catalyzed reactions of hydrocarbons, though they are rarely observed directly. **Bernskoetter *et al.*** (p. 553) glimpse this coordination motif for methane (CH_4) and rhodium (Rh) using low-temperature nuclear magnetic resonance spectroscopy after protonation of an Rh-CH_3 precursor. Kinetics measurements revealed a half-life of just over 80 minutes at -87°C .

A Smooth(ened) Path to Drug Resistance

The Hedgehog (Hh) signaling pathway has emerged as a key contributor to the growth of medulloblastoma, an aggressive brain tumor. GDC-0449, a drug that ramps down this signaling pathway by binding to the Hh pathway component Smoothened, was recently shown to induce rapid and dramatic tumor regression in a patient with metastatic medulloblastoma, but the tumor eventually developed resistance to the drug. **Yauch *et al.*** (p. 572, published online 3 September) show that resistance arose because the tumor acquired a mutation in Smoothened that disrupts binding of the drug. Identification of this resistance mechanism may facilitate the design of next-generation drugs for this type of cancer.

Stretching Carbene Versatility

Stable N-heterocyclic carbene (NHC) molecules are versatile catalysts for organic reactions (see the Perspective by **Albrecht**). **Lavallo and Grubbs** (p. 559) now show that these molecules can also catalyze organometallic transformations. Specifically, the carbenes induce coupling of monomeric iron olefin complexes to form clusters incorporating three or four bonded iron centers. Initial coordination of carbene to iron may facilitate formation of an iron-iron bond with a second complex. In a related development, **Aldeco-Perez *et al.*** (p. 556) prepared an NHC isomer in which the divalent carbon is shifted so that it no longer lies between the nitrogens. The compound forms stable complexes with both gold and CO_2 .



Viral Link to Chronic Fatigue

Chronic fatigue syndrome (CFS) is a complex and debilitating disorder that is often linked to immune system dysfunction but whose cause(s) remain mysterious. **Lombardi *et al.*** (p. 585, published online 8 October; see the Perspective by **Coffin and Stoye**) now present a tantalizing new lead. In blood samples from 101 patients with well-documented CFS, over two-thirds (68) contained DNA from a recently described human gammaretrovirus, xenotropic murine leukemia virus-related virus (XMRV), which possesses sequence similarity to a murine leukemia virus. Cell culture assays confirmed that XMRV derived from CFS patient plasma and from T and B lymphocytes was infectious. Although the correlation with CFS is striking, whether the virus plays a causal role in the disorder remains to be determined. Interestingly, nearly 4% of the 218 healthy donors tested were positive for XMRV, which suggests that this virus—whose pathogenic potential is unknown—may be present in a significant proportion of the general population.

Dissecting Megaenzyme Mechanisms

Filamentous fungi contain a class of multidomain enzymes, the highly-reducing iterative polyketide synthases (HR-IPKSs), which produce important natural products such as the cholesterol-lowering drug lovastatin. To produce their complex products, these megasynthases use multiple catalytic domains repeatedly in different combinations, but mechanistic details remain unclear. **Ma *et al.*** (p. 589) now report in vitro reconstitution of the complete catalytic function of lovastatin nonaketide synthase (LovB), the megasynthase that works together with a partner enzyme LovC to complete nearly 40 chemical steps required to construct the core of lovastatin. Analyses of the dependency of enzyme function on cofactors and on the partner enzyme elucidate the programming rules for this system.

CREDIT: LAVALLO AND GRUBBS

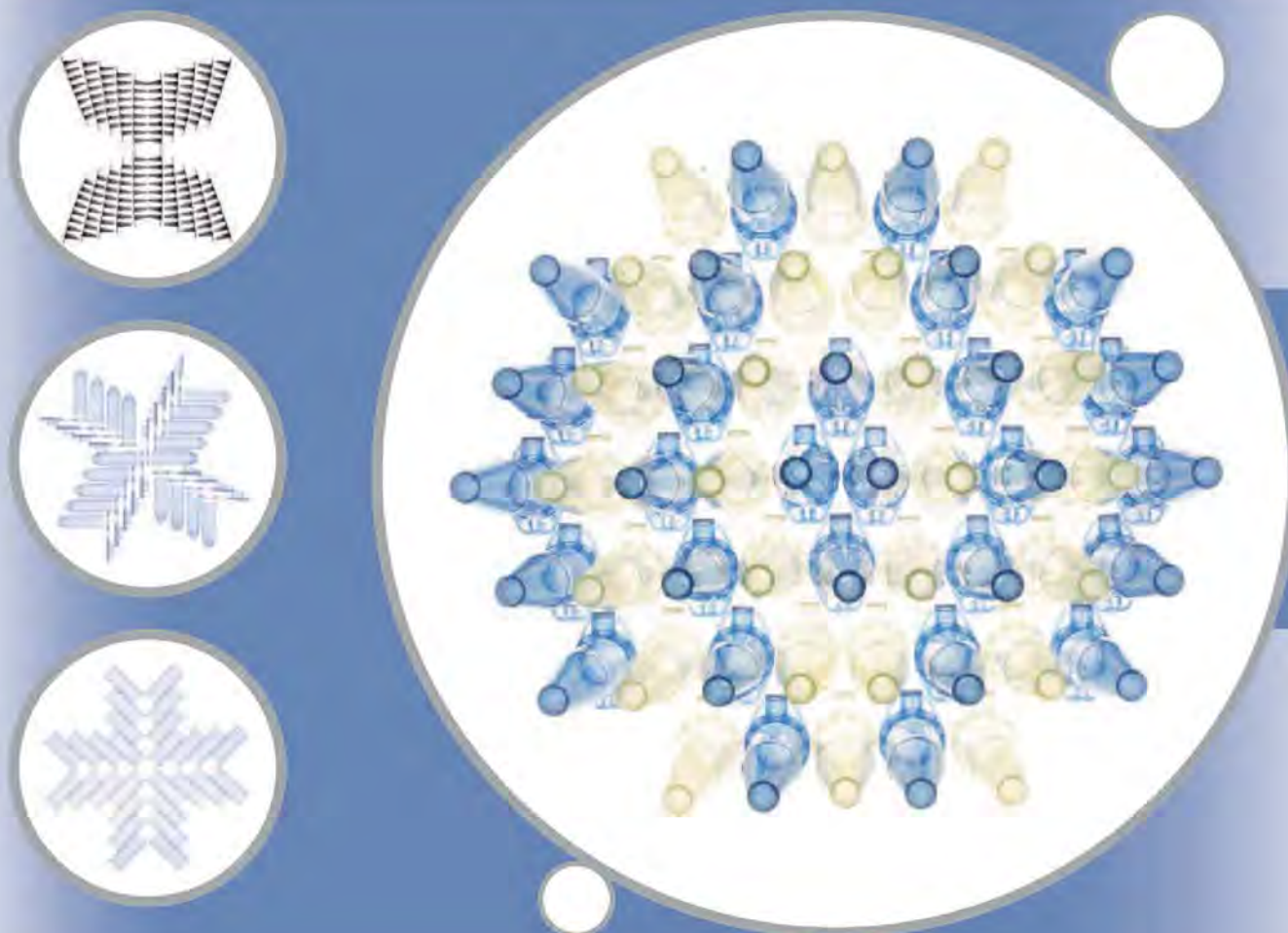


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Europe Rethinks Education

FOR SOCIETIES TO UNDERSTAND THE CONSEQUENCES OF VITAL ISSUES SUCH AS CLIMATE CHANGE, education—especially science education—will play a critical role. Improving the quality of science education in primary and secondary schools is a challenge faced by nearly all countries. Europe has finally recognized the need for a trans-European effort to rejuvenate the scientific education of all students, and promising efforts are now under way.

The Maastricht Treaty of 1992 left formal science education to each nation of the European Union (EU), in contrast to scientific research, which was viewed as a shared trans-European competency. But 2 years ago, Michel Rocard, former prime minister of France and then a member of the European Parliament, submitted an important report to the EU Commission.* Referencing two pilot projects (Pollen in 12 European countries and Sinus-Transfer in Germany), the report advocated that an ambitious program for inquiry-based science education be supported by EU funds of 60 million euros. Last year, a conference in Grenoble on science education, which included scientists, urged the 27 EU education ministers to support this approach. The 60 million euros are now on the way, and the investment is meeting a massive and promising response from the many institutions applying for funds.

In today's economic crisis, the disinterest of European youth in scientific careers and the public's poor understanding of science greatly threaten the future of Europe at a time when science and logical problem-solving skills are critical. Yet the performance of young people from France and Germany on science tests (the Organization for Economic Cooperation and Development Programme for International Student Assessment) is barely average, and many students leave middle school being illiterate in science. Moreover, the content of science education is often questioned, and even disparaged.

Fortunately, the science academies in France, Germany, the Netherlands, and Sweden have been sponsoring exemplary pilot projects that can serve as prototypes for additional efforts. These include the nationwide program *La main à la pâte*, which has brought inquiry-based science to about half of French primary schools. Well-known scientists, such as Georges Charpak, a Nobel laureate in physics, are among those who assist teachers. In all four nations, the "science as inquiry" pedagogy encourages students (ages 5 to 16) to develop a sense of wonder, observation, and logical reasoning. Because of their interactions with scientists, as well as new assessment and professional development methods, teachers gain increased confidence and a better understanding of science as a process.†

Although education remains the responsibility of each nation, the EU open method of coordination promotes an exchange of best practices. The early trans-European Pollen project published guides for practical implementation of inquiry in schools, as well as for teacher training, and it led to a pilot project in Berlin that has expanded to over 100 primary schools in Germany. Pollen (2006–2008) only involved 722 teachers and 15,000 students, but its achievements paved the way for its successor, Fibonacci (2010–2012). With partners in 21 EU countries, Fibonacci expands to middle school and includes mathematics; using Pollen-elaborated tools, it aims to pair expert centers with emerging ones throughout Europe.

The funds generated by the EU's response to the Rocard report can only serve as seed money for creating pilot projects with a common goal. The report's eventual impact will depend on national measures that change how teachers are prepared and supported to teach science, as well as on the continued involvement of the national science academies. Only national education authorities can produce large-scale dissemination of pilot projects and the required improvements in in-service and pre-service teacher training. And only active scientists and engineers can help to convey in classrooms the message that science is a rich human adventure, vital for the future of Europe.

— Pierre Léna

10.1126/science.1175130



*M. Rocard, *Science Education Now: A Renewed Pedagogy for the Future of Europe* (Report EU22-845, Brussels, 2007).

†J. Osborne, J. Dillon, *Science Education in Europe: Critical Reflections* (Nuffield Foundation, UK, 2008).

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EVOLUTION

Not So Useless

For humans, the value of having an appendix seems to be negligible and, given the prevalence of appendicitis, having an appendix can even be dangerous. This gut attachment has long been thought to be a remnant of the time when hominids ate a high proportion of plant matter that needed fermentation before digestion. More recently, the appendix has been proposed to play a role in the immune-mediated maintenance of symbiotic bacteria in the gut. On the basis of comparative anatomical and phylogenetic approaches, Smith *et al.* now contend that the appendix is a specialized organ for harboring symbiotic bacteria essential for health. Diarrhea was a common hazard during hominid evolution. Because the opening to the appendix is constricted, it may escape colonization by bacterial pathogens. Bacterial symbiont reconstitution after diarrhea can be achieved rapidly from the populations harbored in the appendix. Thus, far from being useless, positive selection may well have acted to maintain the appendix. — CA

J. Evol. Biol. 22, 1984 (2009).

PHYSICS

A Cloudy Thermometer

Some of the coldest places on our planet are created in physics laboratories, where dilute alkali gases are coaxed into showing their quantum character. To prepare the samples, experimenters employ irreversible methods such as evaporative and sympathetic cooling. It is often hard to measure the final temperature of the gases, however, particularly in contexts where they are loaded into optical lattices to simulate condensed-matter systems such as Mott insulators and superconductors. Catani *et al.* address this challenge by tracking entropy, rather than temperature (which, technically speaking, is a measure of how much a system's entropy changes on incremental energy input). Specifically, they load two distinguishable Bose gases into the same magnetic trap and then apply an additional optical trap that affects only one of them, the target gas. By slowly increasing the strength of the trap, they achieve an exact entropy transfer from the target gas (^{41}K) to the auxiliary gas (^{87}Rb), condensing the K and maintaining the temperature equilibrium between the

two gases. Through cycles of compression and decompression, they observe oscillation of the K ensemble between condensed and uncondensed states. When the target gas is then loaded into a one-dimensional optical lattice, its temperature is directly reflected in the auxiliary gas cloud size after expansion. — JS

Phys. Rev. Lett. 103, 140401 (2009).

NEUROSCIENCE

Memories of MAP Kinase Activation

We all know from experience that nothing beats repetition to help us store memories. New studies in the fruit fly of a gene associated with Noonan syndrome, which in humans causes abnormal development and learning disabilities, have implicated a role for signaling through the mitogen-activated protein kinase (MAPK) pathway in the consolidation of long-term memories in the brain. Mutations that cause Noonan syndrome are clustered in components of the MAPK signaling pathway, in particular, the gene encoding

the protein tyrosine phosphatase PTPN11. Pagani *et al.* characterized the effects of the *Drosophila* ortholog of PTPN11, called corkscrew (CSW), that promotes MAPK signaling. In a learning task in which repetition of exposure to a stimulus was required to produce long-term memory and in which the time between training events was also critical, genetic or pharmacological inhibition of CSW specifically inhibited long-term memory formation. Overexpression of CSW in brain neurons altered the intervals required between trials. Successful generation of long-term memories was correlated with conditions that allowed a transient peak in MAPK activity after each training trial. Thus, waves of MAPK signaling may be part of the molecular mechanism through which properly spaced repetitions of a task produce long-term memories. — LBR

Cell 139, 186 (2009).

CLIMATE SCIENCE

Dates in a Cave

Stalagmites—one type of a class of secondary mineral deposits, or speleothems, formed in caves—can provide precisely datable records of climate if they formed under the right conditions. Their datability is in fact almost unparalleled in most paleoclimate proxies. Fleitmann *et al.* present a record of climate, derived from stalagmites found in northern Turkey, which covers the past 50,000 years, including the 13 most recent Greenland interstadial (cold) events. The record closely resembles the Greenland ice core record of these episodes. The stalagmites can be dated much more precisely than the ice cores, however, and thus should generate a better ice chronology than



those that exist now. Moreover, the improved age scale for the Greenland interstadial events does not support the idea (based on age models derived from ice cores) that the events occurred with an irregular periodicity of 1500 years, suggesting instead that the events were more likely to be stochastic in origin. — HJS

Geophys. Res. Lett. 36, L19707 (2009).

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TRULY PSYCHEDELIC 'SHROOMS

No, you're not hallucinating: Those mushrooms are glowing. While mushroom-hunting at night in a Brazilian forest, mycologist Dennis Desjardin of San Francisco State University and colleagues discovered *Mycena luxaeterna* growing on sticks. Luminescent chemicals similar to those in fireflies produce the fungus's constant glow—possibly to attract insects to spread spores or eat harmful bugs, Desjardin says. Mycologists have previously identified 64 luminous species from 16 different lineages, suggesting that either luminescence evolved multiple times or most species gradually lost the glow. The scientists describe the new fungus and six others online this month in *Mycologia*.



Andean Gold

During the Spanish conquest of the Andes, conquistadors seized enormous quantities of silver and gold objects. Because archaeologists long believed that Andean societies had almost no metallurgy, they have only recently begun to unravel how these precious metals were produced. Now a team of geologists argues that these indigenous people refined gold with mercury amalgamation, a sophisticated metallurgical technique.

By mixing liquid mercury with finely ground gold or silver ore, metalworkers create an amalgam (alloy). They then separate out the heavier amalgam and heat it to boil away the mercury, arriving at almost-pure silver or gold.

This process was widespread in Europe by

the 12th century but was thought to be non-existent in pre-Columbian America. Now William E. Brooks, a consulting geologist in Reston, Virginia, and colleagues have analyzed residual mercury levels in gold foil from the Sicán culture, which existed between 750 and 1375. The team found signs of amalgamation similar to those seen in contemporary gold foil in southeastern Peru, they reported this week at the Geological Society of America annual meeting in Portland, Oregon. "We think this technique was used throughout the Andes, probably centuries before it was commonly used in Europe," Brooks says. Archaeologist Izumi Shimada of Southern Illinois University in Carbondale cautions that more research is needed because many Sicán artifacts were coated with cinnabar,

a mercury-based pigment, which could contaminate the measurements.

Follow the Sea Lion

Tracking endangered Steller sea lions in Alaska just got easier. Researchers have devised a new long-lived transmitter that reports the moment, location, and even cause of the animal's death.

Satellite-linked transmitter tags usually last about a year. But the new device's 10-year battery life allows it to record the sea lion's body temperature until it dies, says Markus Horning, one of the device's developers. Because researchers surgically implant the transmitter into the sea lion's lower abdomen, a temperature change can suggest its fate: A sudden cooling may mean a shark or killer whale attack; gradual cooling suggests old age, disease, or starvation.

After testing the tags in nonendangered California sea lions, Horning, a pinniped ecologist at Oregon State University, Newport, and colleagues tracked 27 Steller sea lions released into the Gulf of Alaska in 2005. Four years later, five animals have died, four most likely by predators' jaws, the scientists reported this month at the Society for Marine Mammalogy meeting in Quebec City, Canada.

Since the 1970s, Steller sea lion numbers off Alaska have plummeted nearly 80% (*Science*, 4 April 2008, p. 44). The new device should help explain the decline, says research ecologist Lowell Fritz of the National Oceanic and Atmospheric Administration's Alaska Fisheries Science Center in Seattle, Washington.



Run Away!

Marmots in Colorado haven't bumped into wolves for more than 70 years, but a new study reports that they still know what to do: Run.

Faced with a predator, prey animals might freeze, alert others, or dart, depending on the threat. But what if the predator disappears from their habitat? Behavioral ecologist Daniel Blumstein of the University of California,

Los Angeles, hypothesized that responses to different predators evolve together. So if an animal still encounters some predators, it should retain the whole repertoire even when one vanishes.

To find out, Blumstein and colleagues studied 24 wild yellow-bellied marmots. They set up piles of bait 20 meters away from hidden life-size cutouts of predators that marmots see often—red foxes and coyotes—and those they don't: mountain lions and wolves. When a marmot arrived, the researchers uncovered a cutout.

The rodents responded appropriately to each predator, the scientists report this month in *Animal Behaviour*. Mountain lions sneak up on prey, so the marmots made alarm calls to blow the lion's cover. They fled from 2D wolves. "I would too: They're group hunters!" Blumstein says. The results add to evidence that antipredator behaviors can persist after predators disappear, says evolutionary biologist David Lahti of the City University of New York.



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CONSERVATION BIOLOGY

Research Wolves of Yellowstone Killed in Hunt

On 3 October, a few weeks after Montana opened its first legal wolf-hunting season in decades, a hunter killed a female wolf in the Absaroka-Beartooth Wilderness, less than a mile from the border of Yellowstone National Park. She wasn't the first Northern Rocky Mountain gray wolf to be legally hunted since wolves were removed from the federal endangered species list last May. But she was the alpha female of Yellowstone Park's Cottonwood Pack and wore a large radio collar identifying her as wolf 527F. Her behavior, travels, life history, and genealogy had been studied in detail by scientists for 5 of her 7 years. Her death, and that of five other pack members also shot outside Yellowstone, including another radio-collared female, have irrevocably changed what had been a unique long-term study, the researchers say.

"We were studying one of the very few unexploited wolf populations in North America," where packs had lived and died naturally, says wildlife biologist Douglas Smith, leader of Yellowstone's wolf project, which has tracked the wolves since their reintroduction in 1995. "We can no longer make that claim."

The park's wolf project, partially funded by a \$480,000, 5-year National Science Foundation grant, isn't the only scientific study adversely affected. The death of the wolves and loss of the pack are also a blow to a host of studies, from wolf behavior to elk management and ecology, say other scientists, several

of whom have repeatedly asked Montana's Fish, Wildlife and Parks (FWP) department to establish a no-wolf-hunting zone around the park (*Science*, 15 February 2008, p. 890). "Yellowstone is one of the best examples in the world of what happens naturally to an ecosystem when an apex predator is returned," says ecologist William Ripple of Oregon State University, Corvallis, who has shown that wolves are helping to rebalance the park's ecosystem (*Science*, 27 July 2007, p. 438). "If the park wolves are being shot at, they're bound to change their behavior."

A possible buffer zone and other suggestions will be considered as they review this season's hunt, say FWP officials, who add that the hunt that killed 527F had not worked out as expected. In only 4 weeks, hunters had killed nine wolves in the Absaroka-Beartooth Wilderness, including 527F, nearly filling the quota of 12 wolves for this area's early season hunt. As a result, the agency last week closed the wilderness to wolf hunting for the remainder of the season, which ends when snow keeps hunters out.

"We didn't think that wolves would be that vulnerable in the backcountry, so the level of harvest there has been a bit of a surprise," says Carolyn Sime, FWP's wolf program coordinator in Helena, who added that the hunt was designed to target wolves that kill livestock, not wilderness or park wolves that have never caused problems in that area.



Fair game? Wolf 527F (above) was shot by a hunter, which affects researcher Doug Smith's studies.

However, many hunting camps are set up in the Absaroka-Beartooth Wilderness to take advantage of elk migrating out of Yellowstone, conservationists point out. Also, park wolves are naïve. "Every person the park wolves encountered was benign until now," says Smith.

Inside the park, wolves are regarded as study animals and tourist magnets, pulling in a minimum of \$35 million a year in tourist dollars, according to a 2006 University of Montana study. But as soon as a wolf crosses into Montana, it falls under state law, which regards the canids as "a species in need of management"—another big game animal that can be hunted like the deer, elk, bear, and mountain lion that also travel in and out of the park. Five of Yellowstone's remaining 12 packs have territories that stray outside park borders.

Some wildlife officials point out that the Cottonwood Pack may not be completely gone. The killing of most of its members has not greatly harmed Yellowstone's wolves or scientists' research, they argue, because there are more than 100 wolves left in the park and one wolf pack is very like another. "Biologically, [the loss] has no impact, since wolf packs turn over all the time," says Edward Bangs, wolf recovery coordinator for the U.S. Fish and Wildlife Service in Helena. "It doesn't make

CREDITS (LEFT TO RIGHT): WILLIAM CAMPBELL/U.S. FISH AND WILDLIFE SERVICE; NATIONAL PARK SERVICE



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any difference to wolf conservation or wolf research, although it will cost Doug [Smith] more money to collar another wolf."

But from Smith's perspective the Cottonwood Pack is gone, and he will need to collar two more wolves—a dangerous, time-consuming task, costing \$1500 per wolf—to successfully track whatever pack moves into the Cottonwood's former territory, which was 95% inside park boundaries. In addition, much of the data gathered on 527F and her pack are now worthless because the wolves met an unnatural end and no longer fit the project's study criteria, he says. The project is now adding a new category to many of its 85 databases: harvested wolf.

A secretive wolf, whose territory this year was so remote that researchers seldom saw her, 527F was raising her third litter. (The fate of her five 5-month-old pups is not known.) At the

advanced age of 7, she was a key animal in many studies, including some on how long wolves live, their maximum body size, and female wolves' lifetime reproductive success. These are some of the many unknowns of wolf biology that "can't be studied outside Yellowstone because people curtail wolves' maximum life spans," explains Smith.

Smith collars the wolves, but many scientists independent of the wolf project have been gathering data on the radio-collared wolves. "Any time radio-collared animals are lost, it's a huge setback for our research, since it's the best tool for tracking their movements," says Daniel McNulty, an ecologist at Michigan Technical University in Houghton, who has been studying wolf-prey dynamics in Yellowstone.

He worries that annual hunting of Yellowstone's wolves will eventually affect their social dynamics and age structure, "skewing it toward

the younger classes, something that has been demonstrated in every game population" worldwide. That, in turn, could potentially be bad news for the park's elk, because McNulty's research has shown that younger wolves kill more elk. Evolutionary geneticist Robert Wayne of the University of California, Los Angeles, adds that an annual hunt, as is now planned for the Absaroka-Beartooth Wilderness, runs the risk of turning the area into "a predator sink, drawing wolves out of Yellowstone," as young, dispersing animals search for unoccupied territories. "This shouldn't have happened," he says. "Yellowstone's wolves should have absolute protection."

But they don't, and Montana's FWP has a quota of three additional wolves in other areas adjacent to Yellowstone. Montana's statewide wolf-hunting season opens on 25 October.

—VIRGINIA MORELL

AMPHIBIAN DECLINE

Life and Death Play Out on the Skins of Frogs

For herpetologists, the global decline of amphibians has been agonizing. For Jamie Voyles, the most disturbing episode was witnessing the death of diseased frogs at the Omar Torrijos Herrera National Park in the Republic of Panama in 2004. "The subsequent silence left a long-lasting impression on me," recalls Voyles, a graduate student in disease ecology at James Cook University, Townsville, in Australia.

At the time, few clues existed about how the culprit—a fungal infection—could be so lethal. "Understanding how [the fungus] kills frogs was one of the biggest mysteries about the disease," she recalls.

Now on page 582, Voyles and her colleagues go a long way toward solving that mystery. They find that the fungus, *Batrachochytrium dendrobatidis*, causes such severe electrolyte imbalances that the frog's heart stops. "It fills a big knowledge gap about one of the most devastating [amphibian] diseases we've ever encountered," says Brian Gratwicke of the Smithsonian National Zoological Park in Washington, D.C. "This paper clearly points to an osmoregulatory mechanism."

This insight follows other, potentially

promising findings reported in March and in late August that certain skin bacteria can protect against fungal infection. Matthew Becker, now a student at Virginia Polytechnic Institute and State University in Blacksburg, Virginia, and Reid Harris of James Madison University in Harrisonburg, Virginia, and their colleagues have found that the bacterium, *Janthinobacterium lividum*, makes an antifungal compound that stops the fungal infection in its tracks. "This is one of the few bits of hope that many of us have," says Karen Lips, an ecologist at the University of Maryland, College Park.

Herpetologists began realizing there was a worrisome trend in amphibians in 1989. By 2004, a global assessment concluded that "amphibians are more threatened and are declining more rapidly than either birds or mammals" (*Science*, 3 December 2004, p. 1783).



Skin deep. Studies of green tree frogs (inset) revealed how a fungus (seen close up on skin, with tubes for spores) kills amphibians.

At first, researchers blamed habitat destruction or climate change and changes in ultraviolet radiation. Then in 1998, Australian scientists reported finding a strange fungal infection on dead frogs in the rainforest. About the same time, Smithsonian researchers made similar observations in captive frogs, and in 1999, with the help of Joyce Longcore of the University of

Maine, Orono, they described *B. dendrobatidis* and showed that it caused the deadly infections. Of the 120 amphibian extinctions since 1980, about 90 are attributed at least in part to the fungus, says Gratwicke.

Six years ago, Voyles began trying to find out what made the fungus so deadly. She and her colleagues had very little to go on. These fungi weren't known to attack vertebrates, and the "attack" was just skin deep. Moreover, "light microscopy and traditional methods used to determine pathogenesis were ineffective," Voyles recalls.

Two theories held sway. One was that the fungus released a deadly toxin. The other attributed the problems to a disruption in skin function. The skin of amphibians does double duty: maintaining the proper osmotic balance inside the animal and regulating respiration.

In 2007, Voyles and her colleagues showed that blood taken from diseased frogs contained very low levels of electrolytes, causing them to suspect a disruption in osmotic balance. Out-of-whack electrolytes affect many physiological processes, including muscle and nerve function and water balance.

In the lab, the researchers infected green tree frogs and studied skin samples from these and uninfected frogs. They monitored how well the skin transported electrolytes, tracked biochemical changes in the blood and urine, and kept tabs on heart function using implanted transmitters.

The skin of infected frogs was less adept at transporting sodium and chloride ions. Sodium and potassium concentrations in the blood dropped, more so as the infection intensified. The animals' hearts began to beat irregularly and ultimately stopped. When the researchers gave dying green tree frogs electrolyte supplements, frogs that could no longer right themselves recovered enough to turn over and move again. They outlasted the other infected frogs by about 20 hours.

"An important next step for this research is to determine what causes the disruption in osmoregulation," says Becker. Adds Allan Pessier of the San Diego Zoo in California, "It would be good to see these studies repeated and verified in other susceptible amphibians."

In their work with potentially protective bacteria, Becker, Harris, and colleagues have

expanded their studies to multiple species. They had come across this bacterium while studying communal nests of four-toed salamanders. The salamander produces a compound called violacein that's toxic to *B. dendrobatidis*. In March, they reported in the *ISME Journal* that putting this bacterium on the skin of infected mountain yellow-legged frogs averted death from the fungal infection. They have now tested red-backed salamanders. As they described online 28 August in *Applied and Environmental Microbiology*, some salamanders have violacein on their skin already, likely produced by endemic bacteria. But adding more bacteria increased the concentration of violacein.

"The study clearly demonstrated that violacein concentration on amphibian skin can be manipulated by adding symbiotic bacteria," says Doug Woodhams, a disease ecologist at the University of Zurich in Switzerland. "By manipulating the microbial community on amphibian skin, conservationists may be able to enhance disease resistance and prevent catastrophic amphibian declines."

—ELIZABETH PENNISI



CHINA

Scientists Line Up Against Dam That Would Alter Protected Wetlands

XINGZI COUNTY, CHINA—In an annual rite of autumn and winter, millions of migratory birds, including endangered white cranes, descend on Poyang Lake, China's largest body of fresh water. But the vital wintering grounds may be at risk. A proposed dam across Poyang's northern end, 27 kilometers from the Yangtze River, would destroy delicate habitat and impede rare Yangtze finless porpoises from reaching spawning areas, scientists argue. The ecosystem "would change fundamentally," says wetland ecologist Chen Kelin, director of Wetlands International-China.

In an 11th-hour bid to derail the dam, Chen and other scientists outlined their concerns in two letters this past summer to China's premier, Wen Jiabao, chair of the State Council. Their appeal seemed to fall short: On a visit here late last month, Chinese Vice-Premier Li Keqiang endorsed the project's basic direction while stipulating that "the dam needs further scientific input" and that it must protect Poyang's ecology.

But in a surprising development, the State Council ordered critics and the dam's backers—the Jiangxi Province government—to thrash out

For the birds. A dam at Poyang would harm migratory bird habitat, scientists say.

their differences in Beijing. At the meeting last week, scientists argued that the present proposal's costs outweigh its benefits and pressed Jiangxi officials, led by Hu Zhenpeng, the province's vice governor from 1999 to 2008, to shelve plans for the dam, pending further research. "Until now, the project of Jiangxi government is far from being demonstrated as necessary," says Li Wenhua, an ecologist at the Institute of Geographical Sciences and Natural Resources Research (IGSNRR) of the Chinese Academy of Sciences in Beijing. "The government should organize scientists of different research fields to study the project and judge whether to build the dam," adds IGSNRR geographer Sun Honglie. "Balancing ecology and development is necessary."

Poyang is more and more precious, Li says, because many wetlands of the Yangtze River basin are gone. In recent decades, wetlands in the middle and lower Yangtze, upstream of Poyang, "have been seriously degraded" as developers fill them in, says ecologist Lei Guangchun, dean of the nature conservation school at Beijing Forest University. That makes the relatively undeveloped Poyang more critical to preserving ecosystem function, he says.

A section of Poyang is one of seven wetlands that China first protected under the inter-

CREDIT: COURTESY OF HU ZHENPENG

national Ramsar Convention in 1992. One iconic winter resident is the white crane: The 4000 or so individuals that flock here account for 95% of the remaining population, says Zhang Zhengwang, a professor at Beijing Normal University and secretary general of the China Ornithological Society. The big draw for cranes and other migratory birds are Poyang's shallow waters and marshes, ideal for wading and spearing fish with bills.

Proponents argue that a dam would even out Poyang's water fluctuations, which have grown wilder in recent years. During the summer monsoon season, the Poyang watershed, covering 162,200 square kilometers, serves as a spillway for the Yangtze. Lake waters retreat in autumn and winter, and in recent years—perhaps because of the massive Three Gorges Dam upstream—sections have dried up altogether, causing huge losses for fisheries.

Convinced of the economic benefit of stabilizing lake levels, Jiangxi's government in March 2008 aired plans for a dam about 2.8 kilometers long that would keep Poyang flush in winter, when lake water typically drains into the Yangtze. Sluices would remain open from April to October, when the water level is higher, allowing free passage to the Yangtze for fish and porpoises, Hu says. A dam, he and others argue, would both improve water quality and safeguard bird habitat.

Many scientists disagree. "Based on our own research and other available data, [we] concluded that the proposed water-control structure at the outlet to Poyang Lake would have a significant, negative impact on cranes and other wintering water birds," says James T. Harris, vice president of the International Crane Foundation in Baraboo, Wisconsin. Stabilizing lake levels would mean that in winter, "almost all" migratory bird habitat would be lost, Harris says. "The water would be too deep for the birds to access food," he says. "The change in winter water depths would dramatically impact large populations of rare water birds and might lead to the extinction of the Siberian Crane," adds Jeb Barzen, field ecology director at the International Crane Foundation.

In a letter to Wen last June, Chen Kelin and 26 other scientists warned that a dam would "deeply damage" Poyang's ecology and wetland biodiversity. Then in September, 15 senior scientists sent a second letter arguing that stabilizing Poyang's water levels "will trigger a fundamental change of wetland vegetation" and

disrupt the food chain.

In a March 2009 letter to China's foreign affairs minister, Yang Jiechi, the Ramsar Convention's secretariat reminded that treaty parties are required to "maintain the ecological character of all designated Ramsar sites." The secretariat, based in Gland, Switzerland, wondered whether the dam would reduce Poyang's water quality and lead to algal



Not a done deal. Dam proponent Hu Zhenpeng, a top Jiangxi official, says he's open to scientific advice.

blooms, and whether an increased sedimentation rate would erode Poyang's flood storage capacity. "We are concerned as much with the health and livelihood of the human communities that depend on the wetlands, as with ensuring that the function and values of the wetland is maintained for future generations," the secretariat said in a statement to *Science*.

Dam backers argue that such concerns are overblown. The Ramsar-protected wetlands cover only 224 square kilometers, or 5% of Poyang's area, says Sun Xiaoshan, director of Jiangxi's Water Resources Department. "We will protect these wetlands," he says. Hu adds that it doesn't make sense to allow vast areas of Poyang to dry out in winter and says further research is needed to determine the lake's appropriate level and how best to operate the dam. He acknowledges that a dam will reduce flow rates into Poyang and increase eutrophication risk—but this is a problem that managers "can guard against."

Based on the discussions at the meeting, some scientists say the case against a dam is watertight. Others are not sure. "The dam must meet strict environmental criteria," says Xia Qing, an ecologist of the Chinese Research Academy of Environmental Sciences in Beijing. He and others hope that Jiangxi officials at least apply the brakes and involve outside experts in assessing Poyang's future. Adds Chen Zhikai of the China Institute of Water Resources and Hydropower Research in Beijing, "Nobody can evaluate the dam without further research."

—LI JIAO

Li Jiao is a writer in Beijing.

ScienceNOW.org

From *Science's* Online Daily News Site

Black Hole Conditions, Right Here on Earth

A team of researchers has created conditions analogous to those found outside of a black hole by blasting a plastic pellet with high-energy laser beams. The advance should sharpen insights into the behavior of matter and energy in extreme conditions. <http://bit.ly/LAyXH>

Trust Is Only Skin Deep

Good looks can take you far. Strangers rate attractive people as more trustworthy and honest; lookers earn more money, too. Now, scientists report that this deferential treatment may lead the beautiful to be more trusting when they know others can see them. <http://bit.ly/2Y04il>

Radio Waves "See" Through Walls

Researchers have discovered that an array of radio transceivers—devices that send and receive signals—can track people's movements behind walls. Possible uses include detecting people trapped in burning buildings, controlling lighting or heating and cooling systems as people enter or exit rooms, and spotting burglars or enemy soldiers. <http://bit.ly/3wwepw>



Mother's Cancer Can Infect Her Fetus

A startling case in Japan has confirmed that pregnant women with cancer can pass the disease to their fetuses. These transmissions, normally blocked by the placenta, are rare, so the work likely won't change how doctors screen or care for pregnant women. But scientists say the case could help illuminate how cancer foils the body's immune system. <http://bit.ly/1RQmCv>

Read the full postings, comments, and more on scienownow.sciencemag.org.

ARCHAEOLOGY

Archaeologists Alarmed by Turkey's Proposed Dig Rules

Proposed new rules governing foreign-led excavations in Turkey are causing alarm and confusion among archaeologists, who fear that the regulations could make it very difficult for foreigners to dig in the country. The draft regulations, drawn up by Turkey's Culture and Tourism Ministry, would apply to the 43 foreign excavations currently under way and possibly to an additional 34 foreign-led archaeological surveys. They could require that each excavation season last at least 4 months and that a Turkish co-director be appointed for each dig.

A number of foreign governments and archaeological institutes have expressed their concerns to Turkish officials, who met on the issue last week. As a result, a ministry spokesperson now says, the proposed rules may be revised.

Archaeologists are worried because most teach and follow academic schedules. The 4-month rule "would effectively eliminate the

possibility for most U.S. researchers to conduct fieldwork in Turkey," says John Yellen, archaeology program director for the National Science Foundation in Washington, D.C. Nicoletta Momigliano, an archaeologist at Bristol University in the United Kingdom and co-director of a dig at prehistoric Çaltılar in Turkey, says that if her team had to carry out "4 months of actually digging or surveying, it would be impossible to carry on. No academic in the U.K. can afford to have such a long field season."

Turkish officials say the rules were spurred by several issues. Some foreign archaeologists take too long to finish excavations and publish results, says Mesut Özbek, counselor for culture and tourism at the Turkish Embassy in Washington, D.C. "Some of these excavations have been going on for more than 100 years," Özbek says, citing the Austrian-led dig at the classical city of Ephesus on Turkey's Aegean coast. In other cases, he adds, dig "directors

who run out of time do not properly protect their dig sites, and they get attacked by treasure hunters, get looted, or get damaged by nature, rain, and snow." Özbek says that archaeologists should not worry about the 4-month rule, because if they have to leave early, the Turkish co-director can finish the season. "Turkish archaeology is at an advanced stage."

But Sabine Ladstätter, director of the Austrian Archaeological Institute in Vienna, which sponsors the Ephesus excavations, says there are good reasons why the dig there has gone on for 115 years: "The aim of an excavation is not to finish it. This is a huge site, one of the biggest cities of the classical world." Ladstätter adds that the Austrian-led team has carried out numerous restoration projects at Ephesus, most notably the Celsus library, built in 117 C.E. and admired by nearly 2 million tourists each year. She says Turkish archaeologists are fully involved: Of the 174 researchers working at the

SCIENCE IN SOCIETY

Hong Kong's Darwin Defenders Declare Victory in Teaching Fracas

BEIJING—As a year of honoring Charles Darwin and his theory of evolution draws to a close, scientists in Hong Kong are celebrating a partial victory in what is likely to be an ongoing war against proponents of teaching creationism and intelligent design in secondary schools.

"We have kept the creationist barbarians from the gate," says aquatic ecologist David Dudgeon, faculty board chair at the University of Hong Kong (HKU), about a decision last month by Hong Kong's Education Bureau to discount language in new science curriculum guidelines that had opened the door to teaching creationism and intelligent design in secondary schools. But their triumph is bittersweet. The Education Bureau has not revised the guidelines, choosing instead to issue its pro-evolution statement as an annex. And no one expects the few dozen schools in Hong Kong that openly espouse creationism to suddenly abandon it. "It appears that the bureau is unwilling to confront the Christian schools openly, and the schools will probably continue to teach creationism as part of the science classes," says

astronomer Sun Kwok, HKU's science dean.

At first blush, cosmopolitan Hong Kong seems an unlikely bastion of creationism and intelligent design, which posits that the complexity of life requires action by an intelligent agent. But looks can be deceiving. Although all Hong Kong schools are publicly funded, most are run independently, and many have church affiliations, says Kwok. "Fundamentalist Christianity percolates through schools, government, and other authorities in Hong Kong,

and it informs attitudes towards gays and other social issues," Dudgeon says. "It is the elephant in the room" that no one talks about.

That changed in February when Dudgeon, Kwok, and like-minded colleagues began raising a ruckus over the "New Senior Secondary Biology and Combined Science Curriculum and Assessment Guide," a revision aimed at bringing Hong Kong's education system in line with international norms. Many changes were positive, but one rang alarm bells. The previous guidance suggested, vaguely but reasonably, that teachers "guide students to review the differences between scientific theories and other nonscientific modes of explanation, e.g. religious, metaphysical or philosophical." The new wording seems to put religious beliefs on an equal footing with evolution: "In addition to Darwin's theory, students are encouraged to explore other explanations for evolution and the origins of life, to help illustrate the dynamic nature of scientific knowledge."

Some people, Kwok says, perceived that the Education Bureau had "yielded to pressure from religious schools." So Kwok and HKU faculty members mounted a public campaign against what Kwok calls "pseudoscience sub-



In Darwin's corner. The Hong Kong Education Bureau's pro-evolution statement doesn't go far enough, says Sun Kwok.

CREDIT: COURTESY SUN KWOK



Tourist magnet. Visitors flock to the Celsus library at Ephesus, restored by Austrian archaeologists.

site, 46 are from Turkey.

Some Turkish archaeologists have publicly expressed opposition: In July, the Istanbul branch of the Turkish Archaeology Association published a letter on its Web site saying that the obligation to appoint Turkish co-directors could jeopardize “the future of Turkey’s archaeology.” One reason, some researchers say privately, is that Turkey may not have enough archaeologists to go around, as Turkish researchers are busy working their

own sites. Foreign digs, which have long been welcome in Turkey, make up nearly one-third of the country’s roughly 140 excavations. Co-directors are not required in many other countries that welcome foreign archaeologists, including Egypt and Jordan.

After officials from several countries, including Austria, Germany, and the United States, conveyed concerns to the Turkish government, the culture ministry scheduled a meeting with foreign and Turkish archaeologists for 15 October. The week before, the ministry reportedly told foreign archaeologists that it was canceled. However, Özbek told *Science* that Culture and Tourism Minister Ertuğrul Günay did meet with culture ministry officials and Turkish archaeologists; only foreigners were excluded. But there are signs that the foreign archaeologists’ concerns are being heard. According to Özbek and others familiar with the meeting, the ministry plans to hold more discussions before making a decision and is considering including dig preparation and publication as part of the 4-month work period. —MICHAEL BALTER

jects such as intelligent design, astrology, and UFO studies [that] have no place in our science curriculum.” Hong Kong newspapers ate it up. But many religious leaders rallied behind the Education Bureau—as did some members of the scientific community. In May, a group of academics and high school teachers called the new guidance “stimulating, balanced, and nonbiased.” Their statement said that “there is a real legitimate scientific controversy over Darwinian Theory. ... Alternative explanations to Darwinian macro-evolution should thus be explored so long as they are based on rational and empirical grounds.”

One of the signatories, HKU physicist Chris Beling, argues that intelligent design concepts should be taught in addition to Darwinian theory. Intelligent design “may or may not be the answer to present problems in biological origins,” he says, “but if the [HKU] science faculty keeps on shouting that Darwinian theory is the answer and drowning out other voices, it is clearly unhealthy for the progress of science and for the promotion of critical thinking amongst students.”

After weeks of rancor, the Education Bureau sided with the Darwinian camp. In a 9 September letter to the Concern Group for Hong Kong Science Education, curriculum officer Cheung Kwok-wah, writing on behalf of the education secretary, revealed an annex to the curriculum guide that notes,

“Creationism and Intelligent Design are not included in the Biology Curriculum framework nor are they considered as an alternative to Darwin’s theory.”

Kwok, for one, is not satisfied. “The bureau has not changed the curriculum guide or issued a clarification statement to the schools,” he notes. In the meantime, Kwok has been overseeing an effort to strengthen HKU’s science curriculum as the university moves to a 4-year program. The science faculty is designing “foundation courses” that, starting in 2012, he says, would “provide all science students with a broader education and ensure that all students are exposed to the scientific way of thinking.”

Secondary schools are likely to be more resistant to change, however. Members of the Concern Group—now with more than 630 members—recently discovered that one biology textbook published by Oxford University Press (China) Ltd. and endorsed by the Education Bureau refers to intelligent design ideas and two creationist Web sites. Some schools are using it, says information technologist Virginia Yue, a founder of the Concern Group. “We were shocked and appalled by such shameless religious proselytizing under the guise of science,” says Yue, whose group is now mulling its next move. Their options may be limited, however. “Unless we police classrooms,” Dudgeon says, “I think the matter must rest.”

—RICHARD STONE

ScienceInsider

From the Science Policy Blog



Although the number of swine flu cases is rising in the United States, deliveries of **pandemic vaccine** have been delayed. The Department of Health and Human Services, which had hoped to deliver at least 40 million doses by the end of October, is now estimating 30 million or fewer. <http://bit.ly/2WCqCf>

The **FBI** arrested U.S. space scientist **Stewart David Nozette** 19 October on charges of attempted espionage. Nozette had a security clearance at Lawrence Livermore National Laboratory. <http://bit.ly/2JKquC>

Story Landis, director of the National Institute of Neurological Disorders and Stroke, resigned from the Interagency Autism Coordinating Committee. She accidentally left notes that angered **autism advocates**. <http://bit.ly/11TMha>

The **U.S. Congress** will hold its first hearing early next month on proposals to deliberately tinker with the climate. Legislators who support curbing carbon emissions have shied away from **geoengineering** out of concern that talk of a technical fix could distract from those efforts. <http://bit.ly/18jyGz>

An Iranian lawmaker who helped investigate **alleged plagiarism by Iran’s science minister** says that the case isn’t plagiarism because the results are a “genuine scientific work.” Sections of a 2009 engineering paper written by Minister Kamran Daneshjou were verbatim copies of work by other scientists. <http://bit.ly/AmADy>

The **Large Hadron Collider** at CERN near Geneva, Switzerland, is ready to begin smashing particles, officials say. The \$5.5 billion accelerator broke down 13 months ago after operating for only 9 days. <http://bit.ly/2AY9kM>

The **Jackson Laboratory**, the mouse-research powerhouse in Bar Harbor, Maine, is thinking about building a branch in south Florida as part of a move into **personalized medicine**. <http://bit.ly/21syXV>

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Race for The Heavens

Two very different telescope projects are jostling to give the United States its biggest-ever eye on the sky. Can the country afford both?

IN 1977, JERRY NELSON, AN APPLIED physicist at the Lawrence Berkeley National Laboratory in California, made a bold proposal to the University of California (UC). The university was looking to build a 10-meter telescope—twice the size of the Hale Telescope at Mount Palomar, which for 3 decades had been the largest telescope in the country. Nelson was convinced that standard telescope mirrors—“monoliths” made from a single piece of glass—had reached their limits. Instead, he proposed to make the primary mirror for the new telescope from a few dozen thin, hexagonal segments joined together into a smooth parabolic surface.

“I knew I could build a 10-meter monolith. But I didn’t want to, because it would be the last of the dinosaurs,” says Nelson, whose concept was greeted with skepticism by UC astronomers. “I didn’t just want to build a telescope. I wanted to build a system that could be extrapolated into a bigger telescope in the future.”

Meanwhile, a physicist named Roger Angel was melting Pyrex dishes in a makeshift backyard kiln in Tucson, Arizona,

to figure out how to make monolithic mirrors bigger and better. Although less radical than Nelson’s approach, Angel’s posed equally daunting engineering challenges. The two men would exchange competitive jibes at conferences. Making no secret of his view of monoliths as obsolete, Nelson—a native Californian with an irreverent style—would jokingly ask Angel why he kept wasting time on a dead-end technology. Angel, a transplanted Brit of gentlemanly bearing, would smile back and note the risks of an untested one.

By the early 2000s, each side had points on the scoreboard. Nelson’s team had built segmented mirrors for the twin 10-meter Keck telescopes on the summit of Mauna Kea in Hawaii; Angel’s had fabricated 6.5-meter monoliths for the two Magellan telescopes at Las Campanas in Chile. Those successes set the stage for a new contest, now in full throttle: building the world’s largest telescope. For the past 5 years, Nelson and his colleagues at UC have been working on plans for the Thirty Meter Telescope (TMT)—whose primary mirror will be a glinting mosaic of 492 hexagonal segments controlled with such precision

that even light won’t discern the edges between them. Meanwhile, Angel and his collaborators have set their sights on building the Giant Magellan Telescope (GMT)—whose seven monolithic 8.4-meter mirrors, arranged like flower petals, will function as a primary mirror 24.5 meters in diameter.

If the telescopes are built—TMT on Mauna Kea in Hawaii, GMT at Las Campanas—each will capture images up to 10 times sharper than today’s best ground-based telescopes. Both will shoot for the same scientific goals, which include bringing into focus the first stars and galaxies, studying the formation of planets and stars, understanding the growth of black holes, and probing the nature of dark matter and dark energy. And both will cost a fortune: The segmented TMT’s price tag is \$1 billion; GMT’s is \$700 million.

So far, neither side—UC and the California Institute of Technology for TMT, and a consortium led by Carnegie Observatories and the University of Arizona for GMT—has come close to securing the total funding it needs. “These facilities are so big that they could die of their own weight,” warns Richard McCray, a professor emeritus at the University of Colorado, Boulder. Even if both GMT and TMT get built with little federal support, he says, the U.S. National Science Foundation (NSF) would be hard pressed to help out with the substantial operating costs of each. Given the funding challenges, some astronomers say

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S Podcast interview with author Yudhijit Bhattacharjee.

CREDITS (TOP TO BOTTOM): COURTESY OF TMT OBSERVATORY CORP.; GIANT MAGELLAN TELESCOPE/CARNEGIE OBSERVATORY

Rivals. Artists' conceptions of the Thirty Meter Telescope (left) and the Giant Magellan Telescope.

the two sides should have joined hands to build one telescope to rival the European Southern Observatory's proposed 42-meter segmented-mirror telescope, the European Extremely Large Telescope (E-ELT), which is also in the works (see sidebar, p. 514).

Such a merger has certainly crossed the minds of key figures on each side, but everyone agrees that it's too late now. Differences in technology, personal egos, and institutional rivalries have driven an insuperable wedge between the two efforts. "This is a human endeavor," says Nelson, adding that to him, the two projects "look like oil and water." But even though that divide implies an arduous task ahead for both GMT and TMT, along with the risk of delays or failure should funds run short, leaders of both TMT and GMT project absolute confidence that their artists' conceptions, videos, and miniature models are destined to become the real thing. And officially, at least, the two sides conjure up a future in which both telescopes coexist. "Let two flowers bloom, I say," says Angel.

Worlds in collision

Nelson and Angel are both stars, now in their 60s, with gray hair and membership in the National Academy of Sciences. Their different backgrounds and contrasting personalities in some ways mirror their approaches to telescope building: radical versus traditional, new-worldly and risky versus more tried and true.

The son of a Lockheed tool planner and raised in a rural California town, Nelson grew up a tinkerer with an affinity for electronics. He learned lathing, welding, and polishing at the machine shops of Caltech while receiving an undergraduate degree in physics. Nelson has a cheerful, round face and a prosperous middle, likes to wear Hawaiian shirts, ends his e-mails with "Aloha," and grins widely when he's mocking the competition. Student-like, he often carries a backpack slung across his shoulder.

Working on the Keck design 30 years ago, Nelson had to solve two main challenges in constructing the segmented mirror. One was polishing each mirror segment into an aspherical surface, so that the segments would together make a dish. The other was controlling the segments precisely to make them act as one seamless reflector.

Standard polishing renders surfaces spherical. To get around the problem, Nelson—along with Coby Lubliner, a civil engineer at UC Berkeley—prestressed each piece with

weights before polishing it into a spherical surface. When the weights were removed, the segment elastically relaxed into the desired shape.

Nelson and his colleagues solved the control problem with electronics and computing, using edge sensors and pistonlike actuators to keep the segments perfectly positioned against the destabilizing effects of wind, gravity, and temperature change. It was a scheme that many considered too complex to work. "In 1980, control systems were like, 'How do you spell that?'" to the astronomical community," chuckles Nelson. Clearing such practical hurdles, he says, was key to moving the once-outlandish idea of segmented mirrors into the astronomical mainstream. "The world is full of dreamers who say, 'Gee, I'll get in my car, I'll fly to work, then I'll go under water.' The question is, do you know how to do it?"

Angel grew up in foggy climes than Nelson did—in a suburb of Manchester, U.K.—but like his competitor, he studied physics in college and spent time at Caltech on a master's degree. After earning a doctorate from the University of Oxford, U.K., he flitted between different fields of high-energy physics and astrophysics before settling on optics and mirror design as a young faculty member at the University of Arizona. Angel is slim, bespectacled, and more reserved than Nelson. He seems to fit the mold of the absent-minded professor, asking students for help in turning on the lab coffeemaker and driving an old truck through Tucson wearing

"It's a matter of the devil you know versus the devil you don't."

—ROGER ANGEL,
UNIVERSITY OF ARIZONA, TUCSON



"The world is full of dreamers. ... The question is, do you know how to do it?"

—JERRY NELSON, UNIVERSITY OF CALIFORNIA OBSERVATORIES



a straw hat and gloves to shield himself against the merciless Arizona sun.

Angel's innovation in the early 1980s was to design monolithic mirrors that weighed one-fifth as much as conventional mirrors, allowing them to be cast in larger diameters, and that cooled more quickly at night, which would reduce the image distortion caused by air turbulence at the mirror surface. The trick was to melt cheap borosilicate glass into a mold with hexagonal columns made of heat-resistant foam. Spinning the cast spread the glass into a saucerlike shape, with some of the glass trickling down to fill up the empty space between the foam columns. Once the glass solidified, the foam was washed away, producing a thin, smooth top supported by a hollow, honeycombed base a few inches tall. To polish the surface, Angel and his colleagues built a computer-controlled precision lathe that ground down the glass to nanometer-level accuracy.

In the 1980s, Angel set up a warehouse-sized laboratory under a wing of the university's football stadium to make and test these mirrors. The lab in recent years has delivered two 8.4-meter mirrors for the Large Binocular Telescope on Mount Graham in Arizona, which began doing science in 2007. The seven-part GMT mirrors will have a more complex topology than the binocular mirrors, Angel says, but each will be the same size they were and can be made with the same proven technology. His team recently cast the first 8.4-meter mirror for GMT and is now polishing it.

"With the making of the first segment, we have pretty much retired the risk of how you put the telescope together," he says. He has come home in the middle of the day to tend to his ailing cat. Sunlight bouncing off a pool in the back dances on his face as he peels a pomegranate at the lunch table. Making the 492 segments of the competitor TMT work together, Angel says, is inherently riskier than GMT's seven-piece design: "It's a matter of the devil you know versus the devil you don't."

Caution and thrift come up a lot when members of the two teams pitch their projects. "If you look at the two telescopes, you say

that [TMT] is really sexy; this [GMT] is old-fashioned stuff," says Jonathan Kern, an engineer with GMT. "But *this* is old-fashioned for a reason. We are not trying to do anything that hasn't been done, that we can't put a cost on."

Nelson, who otherwise enjoys the label of risk-taker, stresses that segmented mirrors are now a rock-solid technology, too. Controlling 492 segments (inconceivable when the 36-segment Keck mirror saw first light 16 years ago) is "completely trivial" for modern computers, he says. "The bottom line is that today segmented mirrors are cheaper per square meter than monoliths,"

and the savings on mirror construction far outweigh the cost of more-powerful computing and denser actuators. The American telescope projects' transatlantic rival, the E-ELT, will use 1000 segments, Nelson notes. "Nothing can stop a good idea," he says with a grin.

Winner take all?

The race for telescopic supremacy began in 1999, when astronomers' decadal survey—an official rank-ordered "wish list" of proposed projects that researchers submit to the government—cited a giant telescope as a top priority. Nelson's group was first off the block, with a proposal for what was then called the California Extremely Large Telescope (CELT).

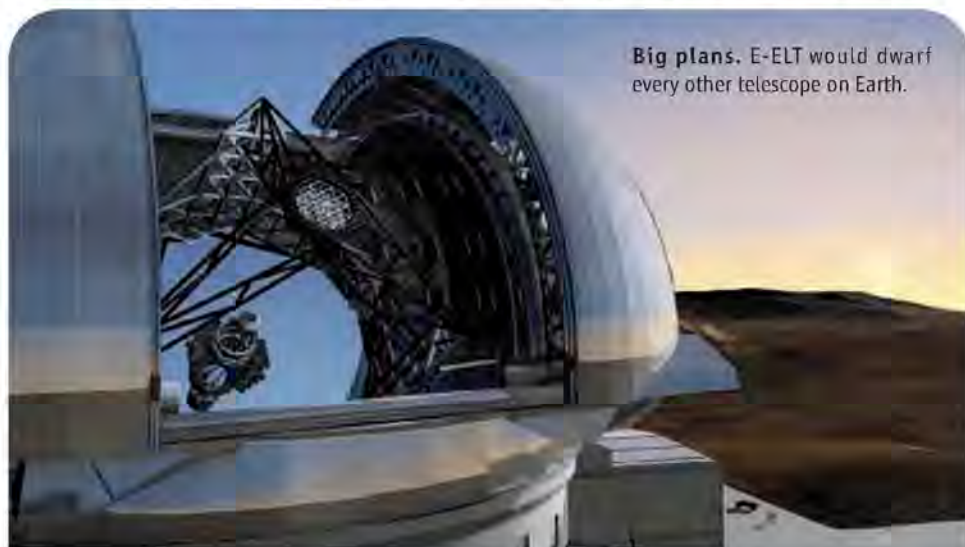
At first, the leadership at Carnegie Observatories mulled the idea of joining CELT. It was a controversial proposal. Carnegie Observatories and Caltech, both nurtured by George Hale and located 8 kilometers apart, had been managed jointly until 1971, when differences culminated in a divorce. Rivalry ran deep.

When Carnegie researchers approached astronomers at Caltech with an offer to collaborate on CELT, they were turned away, according to scientists on both sides who did not wish to spell out the details. Stung by the rebuff, researchers at Carnegie joined with other institutions to create what is now Angel's GMT consortium. "No question that we got going because the other group was making headway," says Stephen Szechtman, project scientist for GMT and a researcher at Carnegie. Nelson and others on TMT acknowledge that they were less than thrilled to see GMT entering the arena. "They wanted to be the only U.S. project in this area," Szechtman says.

Behind-the-scenes maneuvering followed. In 2003, TMT sent UC Berkeley astronomer Richard Ellis to the Harvard-Smithsonian Center for Astrophysics, a Magellan partner, to woo Harvard away from GMT and into TMT. Josh Grindlay, a Harvard University researcher who was involved in the talks, calls Ellis's visit "a political move ... to squash the competition." Nelson is unapologetic. "Harvard's rich. We needed rich partners," he says.

Grindlay says some at Harvard were tempted but that loyalty to the Magellan consortium and confidence in the GMT proposal—"which really is simpler"—carried the day. Friends at Caltech still jokingly ask him if Harvard would like to join TMT, he says: "They say you might as well join us because there may not ever be a GMT."

Nelson's TMT scored a coup in June 2003 when it signed a technical agreement with a major users' group. The Association of Universities for Research in Astronomy (AURA),



Big plans. E-ELT would dwarf every other telescope on Earth.

THE COLOSSUS OF EUROPE

With a 100-meter primary mirror, it would have been the big daddy of all telescopes, worthy of the label "Overwhelmingly Large" (OWL) bestowed by its architects at the European Southern Observatory (ESO). Now, astronomers joke that the acronym means "Originally Was Larger." Even so, the scaled-down successor that ESO has committed to building—the European Extremely Large Telescope (E-ELT)—would still outsize the U.S. entries in its class, the Thirty Meter Telescope and the Giant Magellan Telescope (see main text), by a fair margin, with a primary mirror 42 meters in diameter.

E-ELT's estimated cost of \$1.5 billion also makes it the most expensive of the three. Its funding prospects, however, seem rosier than those of GMT and TMT because governments are backing it: The 14 member states of ESO have agreed to provide a third of the money over the next 10 years. To make up the difference, ESO members are discussing whether to increase their contributions to the ESO budget, attract outside partners, or do both, says Jason Spyromilio, director of the E-ELT project office.

The project's planners, who are currently finishing up a detailed design, want to build the primary mirror with 1000 segments about the same size as the hexagonal panels in TMT. Spyromilio says using a handful of larger monoliths between 7 and 8 meters in diameter could also have worked. "There is no a priori clear solution that would say one is better than the other," he says of the two designs. "The selection reflects the different risks that each project associates with its supply chain."

The resolution provided by E-ELT's collecting area of 1200 square meters—nearly twice that of TMT and three times that of GMT—will enable a lot of exciting science, says Spyromilio. "A nice example might be the power of imaging exoplanets. Here the E-ELT will achieve a contrast about an order of magnitude better than the next best telescope," he says, adding that E-ELT in principle will be able to detect exoplanets as small as Earth. Officials hope to pick a site for the telescope from among candidate sites in the Canary Islands, Chile, Morocco, and Argentina by the end of this year. —Y.B.

THE THIRTY METER TELESCOPE (TMT)

THE TWO TITANS

THE GIANT MAGELLAN TELESCOPE (GMT)

PARTNERS: University of California, Caltech, Association of Canadian Universities for Research in Astronomy

PRICE TAG: \$1 billion

PRIMARY MIRROR: 30 m (492 hexagonal segments, 1.44 m each)

ADAPTIVE OPTICS (AO): Special instrument outside telescope

LOCATION: Mauna Kea, Hawaii

FIRST LIGHT: 2018

FUNDING STATUS: \$200 million from the Moore Foundation; \$100 million pledged by UC, Caltech; commitment by Canada to be a 25% partner; contribution expected from Japan

PARTNERS: Carnegie Observatories, University of Arizona, Harvard, Smithsonian Astrophysical Observatory, Texas A&M, U Texas at Austin, Australian National University, Astronomy Australia Ltd., Korea Astronomy and Space Science Institute

PRICE TAG: \$700 million

PRIMARY MIRROR: 24.5 m (7 monoliths, 8.4 meters each)

ADAPTIVE OPTICS (AO): Integrated into secondary mirror

LOCATION: Las Campanas, Chile

FIRST LIGHT: 2018

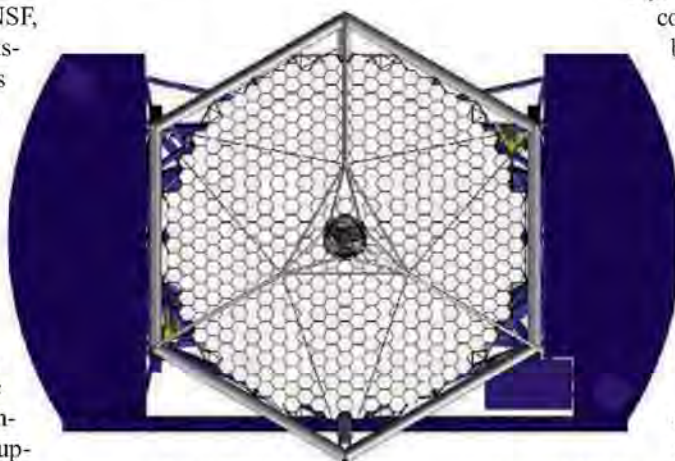
FUNDING STATUS: \$72 million from Australia; > \$120 million pledged by other partners

a consortium of some three dozen U.S. and international institutions that channels NSF funding to astronomers and facilitates access to observatories, nailed down terms of collaboration. The partnership seemed to bring TMT one step closer to NSF funding. Angel and his colleagues fired off a letter to AURA complaining that it was essentially picking a winner without an open competition.

In late 2006, on the advice of NSF, the TMT-AURA partnership was suspended. AURA has since served as the program manager for all technology development related to giant telescopes and has so far provided GMT and TMT with about \$17.5 million each for design work. However, TMT is still lobbying AURA to resume the partnership, arguing that it is the more developed of the two projects and deserves exclusive NSF support. "[TMT's] board is concerned that NSF funding may not support timely substantial public participation in two US-led large telescopes and urges AURA ... to examine carefully the merits and feasibility of advocating support for two large US telescopes in the context of the upcoming Decadal Survey," TMT board chair Henry Yang wrote in a letter to AURA in April 2008. The 2010 survey is expected to be completed by the middle of next year.

NSF officials won't comment on which project is ahead or what its plans are for supporting either telescope in the future. But it is keeping tabs on the progress of both. This summer, a panel appointed by AURA submitted community assessments of TMT and GMT to NSF. The reports do not go into a comparative analysis, but they suggest that TMT is further along. "We believe [TMT] will be ready within a few months for an

NSF-led Preliminary Design Review, and could seek funding assistance from the NSF MREFC [Major Research Equipment and Facilities Construction account] in the near future," the panel writes. GMT, by contrast, needs to make "significant progress" in a number of technical and managerial areas to reach the same stage.



Compound eye. TMT's 30-meter mirror would consist of 492 tiles guided by computer to maintain a smooth parabolic surface.

Wendy Freedman, director of the Carnegie Observatories and chair of the GMT board, acknowledges that the projects are at different stages but says the gap between them is more apparent than real. "We've concentrated on different things," she says. "They have completed their detailed design study, while we've cast the first mirror."

TMT also took an early lead in fundraising thanks to a \$200 million gift from the Gordon and Betty Moore Foundation and a pledge by UC and Caltech to come up with another \$100 million. "When you have a big pile of cash on the table, it shows that somebody has confidence that the thing is going to get built," says Michael Bolte, a UC Santa Cruz astronomer and a TMT board member.

Canada has pledged 25% toward construction and operation, and Japan is interested, he says.

GMT has been working the international circuit as well. In July, the Australian government promised \$72 million for the project. Korea is a partner, too, although the details of how much it would contribute are not available. "We're about a third of the way there," says Patrick McCarthy, director of the GMT consortium. But he is reluctant to provide a breakdown. Both sides have been aggressively courting China and India, McCarthy says, noting that E-ELT officials have been making similar trips.

Amid the scramble for funding and favor, one question looms: Is the game really winner take all? Does NSF have to choose one telescope at the end of the day?

Freedman believes it does not. One possibility, she says, would be for the agency to provide 25% of operating costs to both and guarantee a quarter share of observing time on both for the U.S. community. Furthermore, Angel says, differences between the two projects could lead to different kinds of science. TMT will scan the northern sky, GMT the southern; TMT's adaptive optics system may be better at resolving point sources, whereas GMT's system might produce better images of wider fields, making it more appealing for cosmology.

Nelson takes a hard line. "TMT is inevitable," he says. "I am just doing my job, making it happen." But even he won't entirely rule out a two-scope solution. "If you go back to 1980, you could easily have said, 'Oh, these telescopes are so expensive, they'll never get built'—and lo and behold, there are several 6- to 10-meter telescopes today that back then were the tiniest glint in a few people's eyes." He pauses, then adds: "So, maybe we'll build both."

—YUDHIJIT BHATTACHARJEE



GREEN ENERGY

Another Biofuels Drawback: The Demand for Irrigation

New U.S. mandates are prompting farmers to plant more corn in areas of the country that require irrigation. The move could trigger water shortages and water-quality problems

At first blush, it's easy to make the case for biofuels. By converting crops into ethanol or biodiesel, farmers can reduce demand for imported oil, lower national dependence on authoritarian governments in the Middle East, and potentially cut greenhouse gas emissions.

But dig a little deeper, and the story gets more complicated. Biofuels promise energy and climate gains, but in some cases, those improvements wouldn't be dramatic. And they come with some significant downsides, such as the potential for increasing the price of corn and other food staples. Now, a series of recent studies is underscoring another risk: A widespread shift toward biofuels could pinch water supplies and worsen water pollution. In short, an increased reliance on biofuel trades an oil problem for a water problem.

"It really means a greater potential for agricultural pollution of the waterways, eutrophication of the Gulf Coast, and a significant increase in water use, which may produce localized shortages," says Pedro Alvarez, an environmental engineer at Rice University in Houston, Texas. But just how severe such shortages could become remains unclear. They could be mitigated, some researchers suggest, by steady

improvements in crop yields and an increasing reliance on nonfood feedstocks for making ethanol.

In 2007, the perceived benefits of biofuels helped spur the U.S. Congress to pass the Energy Independence and Security Act (EISA), which mandated a nearly fivefold increase in U.S. ethanol production, to 117 billion liters, by 2022. Of this amount, nearly half is slated to come from corn ethanol by

Thirsty fuel. Corn ethanol is expected to require trillions of liters of additional water by 2015.

2015. Most of the rest will be added from cellulosic ethanol, which is made from agricultural wastes and other feedstocks. Biodiesel and other "advanced biofuels" will eventually chip in about 10%. Although cellulosic ethanol and other advanced biofuels aren't yet cheap enough to compete with corn ethanol, their prices are expected to decline over the next several years.

Growing all those feedstocks will be a stretch, especially for irrigated lands. Meeting the 2015 mandate alone would require 44% of the corn produced in the United States in 2007. That's in addition to existing food and feed corn needs, assuming no change in demand or prices. According to a report in January by May Wu and colleagues at Argonne National Laboratory in Illinois, 98 liters of irrigation water are required on average to produce a liter of corn ethanol. (Estimates range between 10 and 324 liters of water per liter of ethanol, depending on where the corn is grown.) Together, the need for more corn and the mandates for biofuels could increase water demand by some 5.5 trillion liters a year.

Making matters worse, other U.S. energy sectors are growing and increasing their demand for water. Another recent report from Argonne by Deborah Elcock, an energy and environmental policy analyst, for example, found that water consumption for energy production in the United States will jump two-thirds between 2005 and 2030—from about 6 billion gallons of water per day to roughly 10 bgd—driven primarily by population growth. About half of that increase will go toward growing biofuels. According to Elcock's

analysis, which is scheduled to be published in the *Journal of the American Water Resources Association*, the impact from the increase in biofuels is likely to fall almost entirely on the Corn Belt states ranging from the Dakotas, Nebraska, and Kansas eastward to Ohio.

A separate analysis by Charlotte de Fraiture and colleagues at the International Water Management Institute in Colombo, Sri Lanka, underscores the scale of the change. De Fraiture and her colleagues looked at how the expected increase in biofuels would affect countries around the world. They found that the proportion of irri-

WATER REQUIREMENTS FOR ENERGY PRODUCTION (Liters per megawatt hour)

Petroleum Extraction	10-40
Oil Refining	80-150
Oil shale surface retort	170-681
NGCC* power plant, closed loop cooling	230-30,300
Coal integrated gasification combined-cycle	~900
Nuclear power plant, closed loop cooling	~950
Geothermal power plant, closed loop tower	1900-4200
Enhanced oil recovery	~7600
NGCC*, open loop cooling	28,400-75,700
Nuclear power plant, open loop cooling	94,600-227,100
Corn ethanol irrigation	2,270,000-8,670,000
Soybean biodiesel irrigation	13,900,000-27,900,000

*Natural Gas Combined Cycle

THE PROMISE OF DROUGHT-TOLERANT CORN

The biofuels drive is just one of several factors, along with worldwide population growth and rising incomes, that's increasing the demand for corn and other crops. According to a report this year by the Food and Policy Research Institute at Iowa State University, Ames, demand for grains—primarily corn—is expected to grow over the next decade by about 15%, or roughly 200 million metric tons per year. That will put additional stress on global supplies of fresh water, 70% of which already goes to agriculture.

Growers have traditionally responded to increasing demand by boosting crop yields, bringing more land under cultivation, or both. But now seed companies are closing in on a third option: creating seed varieties able to tolerate drought. According to Winwei Xu, a plant geneticist with a joint appointment at Texas Tech University and Texas A&M's Texas AgriLife Research in Lubbock, such drought-resistant varieties could be particularly useful in reducing water demand in areas dependent on declining groundwater reserves. "The use of drought-tolerant and high-yield corn hybrids is key for sustainable corn production under limited irrigation," Xu says.

In traditional corn varieties, even a short drought at the wrong time can spell disaster. Corn seeds are properly pollinated if the pollen emerges from the plants at the same time as the corn silks, which capture the pollen and carry it down to individual corn kernels. But if a dry spell comes just as the plants are flowering, the silks may be delayed—drastically reducing the number of fertilized kernels. Drought that comes a

little later, as corn grains are beginning to fatten, can also sharply limit growth.

Researchers at Monsanto say they have found a way to blunt some of these effects. Earlier this year, they announced that in field trials, newly engineered corn varieties were largely able to maintain yields under drought-like conditions. The Monsanto corn was engineered to express a cold shock protein from the bacterium *Bacillus subtilis*. According to Mark Lawson, who heads corn stress and yields research at Monsanto in St. Louis, Missouri, the protein helps the plant manage stress and continue growing. In early field trials, the extra gene helped boost plant yields by 6% to 10%. Monsanto has applied for regulatory approval of this variety, which should be available beginning in 2012 if all goes well, says Lawson, who adds that the company is also working on a second-generation drought-tolerant corn. DuPont's Pioneer Hi-Bred and other agribusiness companies are also gearing up to release drought-resistant corn.

Whether these new varieties can actually lower water use remains to be seen. Lawson says the goal of first-generation drought-tolerant corn is just to maintain yields during extended dry periods. But Michael Ottman, a crop scientist at the University of Arizona, Tucson, notes that farmers don't like dancing on the edge. For regions such as Nebraska that typically supplement rainwater with irrigation water, Ottman doubts that farmers will cut back on irrigation: "Most irrigation farmers don't want to stress their crops and reduce their yield. So it's hard to imagine they will use less water unless the irrigation district limits it." As a result, even if drought-tolerant corn helps prevent crop losses, it may not actually reduce water use.

—R.F.S.



Hearty stalk. Monsanto's engineered corn (right) resisted drought better than conventional corn plants in a trial.

gation water used to grow biofuels was expected to rise from about 2% to roughly 4% worldwide by 2030. But in the United States, the proportion of irrigation water going to biofuels is expected to skyrocket from about 3% in 2005 to 20% in 2030.

So what's the likely impact from this potential increased water use? That depends on where you are, Wu and others say. "Overall, I think there will be some shortages of water introduced by the EISA mandates," Alvarez says. The potential for shortages is greatest in the western plains states, where average rainfall isn't sufficient to grow corn and biofuel production is increasing, Wu reports. The demands could be particularly challenging for siting biofuel processing facilities. A typical facility might produce 100 million gallons of ethanol a year and use as much water as a town of 5000 people would. Although that amount of water isn't a lot on a national scale, "this can strain local resources," Wu says. "Water is like politics," adds Michael Ottman, a crop scientist at the University of Arizona, Tucson. "It's local that counts."

Another local impact that could hit hard is eutrophication in the Gulf of Mexico, caused by runoff of nitrogen fertilizers into the Mississippi River. Marine ecologists

already see the runoff as a primary culprit in the vast "dead zone" that starves the northern Gulf waters of oxygen, killing everything from crabs to shrimp. A report by Michael Griffin of Carnegie Mellon University in Pittsburgh, Pennsylvania, and colleagues in the 15 October issue of *Environmental Science and Technology* found that even if all the future increase in biofuels were to come from cellulosic feedstocks, the amount of nitrogen pollution in the Gulf of Mexico would continue to rise. That stark forecast illustrates how difficult this environmental problem will be to reverse.

Experts see a few points of light in the gloom, however. Ottman notes that as biofuel production increases, other agricultural water needs may decline. In fact, even as the amount of U.S. land devoted to irrigating corn has grown in recent years, the total amount of irrigated land dropped from 2000 to 2007, as farmers took some land out of production and shifted away from some irrigation-intensive crops such as cotton.

Other factors may curb future water demands. Monsanto and other seed companies, for example, are engineering novel drought-tolerant corn strains that maintain their yields through extended dry spells (see sidebar). And even with current strains, the

amount of water needed to grow and process corn ethanol has been dropping in recent years, thanks to increased yields and improvements in processing technology, according to Wu. The amount of water required to produce a liter of ethanol dropped from 112 to 98 liters between 1998 and 2006, Wu reports. The upshot, Wu, Ottman, and others say, is that better methods will partly offset the increased irrigation demands for biofuels.

The potential impact of cellulosic biofuels remains unclear. Many celluloses will essentially be neutral from a water perspective, says Martha Schlicher, who heads biofuels development at Monsanto. Corn husks, for example, are a byproduct that can be harvested without additional demand for water. And processing switchgrass grown in rain-fed areas requires only between 2 and 10 liters of water per liter of ethanol, according to Wu's report. But Alvarez and others note that as the market develops for cellulosic feedstocks, farmers will have an incentive to begin irrigating switchgrass and other cellulosic ethanol crops to maximize their yields. That could further obscure what has already become a murky case for the advantage of biofuels.

—ROBERT F. SERVICE



BEHAVIORAL ECOLOGY

Sex and Social Structure

Social roles in bees may recapitulate the reproductive life cycle of solitary females

Highly social insects are an efficiency expert's dream come true. Without the benefit of instruction manuals and worker training classes, honey bee queens focus on laying eggs, leaving the details of brood care to an army of workers. One group of workers tends the nest, another forages for food, and neither pays much mind to reproducing. Ants and termites have similar divisions of labor.

As Charles Darwin developed his ideas about evolution and natural selection, he struggled to understand the forces that determine the distinct roles that hive and colony members play. More than a century later—and despite having a much better grasp of genetics—Darwin's successors are still trying to explain how individuals with basically the same genetic makeup can take on such different jobs and appearances.

Now, a series of studies of honey bees by Robert Page and Gro Amdam of Arizona State University (ASU), Tempe, has shown that reproductive traits help shape a honey bee worker's role in life and that ovaries are active players in the process—even if they play little role in reproduction in worker bees. The specialized tasks have their basis in what Amdam and Page call a reproductive ground plan.

"The hypothesis has fundamentally changed the view on the physiological and molecular underpinnings of division of labor in honey bees," says Klaus Hartfelder of the University of São Paulo in Brazil. There was a time when researchers suspected that special genes led to worker-specific behavior. But now, instead of thinking in terms of individual genes, "we have come to look at this problem in terms of regulatory circuits" that govern reproductive physiology in both solitary and social insects.

Although there is debate about the relevance of the work to social insects other than bees—and at least one study has produced contrary data—the idea is gaining attention. "I

think this [idea] has tremendous power to fuel a synthesis of genomics, evolution, and behavior to understand the details of social structures and social evolution," says Michael Breed, who studies the behavior and ecology of social insects at the University of Colorado, Boulder.

Ovarian ground plan

The general idea came first from wasps. In honey bee colonies, the division of labor is fixed, with each bee's destiny as queen or worker determined during larval development. But social wasps can be jacks of all trades. Wasp females play queen, nurse, forager, and guard—depending on the circumstances. To Mary Jane West-Eberhard, an evolutionary biologist at the University of Costa Rica in San José, these different roles were reminiscent of the life-cycle stages solitary female wasps pass through. A solitary female produces and lays an egg; then she spends several days provisioning or guarding the larva before producing another egg and repeating the cycle.

In the late 1980s and again in 1996, West-Eberhard laid out her theory that an "ovarian ground plan" lies behind the behaviors of both social and solitary wasp species. The cycle of egg laying, nurturing, and foraging in solitary wasps is governed by the degree of activation of the ovary, and West-Eberhard suggested that social structure arises when parts of this ovarian cycle are suppressed in some nest members, resulting in the division

of labor between egg laying, nurturing, and foraging that characterizes the social organization of the nest. "Workers drop egg laying, and queens drop (or greatly reduce) brood-care activities," she explains.

While West-Eberhard was developing her ideas, Page was embarking on experiments that would eventually fill in molecular details of how such a ground plan seems to work in honey bees. He didn't set out to do that. In 1990, when he was based at the University of California, Davis, Page began breeding two strains of honey bees, one with workers that had a yen for hoarding pollen and one in which workers tended to collect and store nectar. He knew that the amount of stored pollen available influenced hive dynamics, individual behavior, and other aspects of the colony, and he cataloged the behaviors and traits that emerged



Ovarian cycle. Changes in ovary status cause young *Polistes* wasps to lay eggs when young (right) but forage when older (left).

over time in the two strains. "I wanted to ... see how [the experiment] affected elements at [all] levels of organization," he recalls.

A dozen years later, he had observed several key differences in these strains but couldn't figure out why certain behaviors seemed to be correlated or what underlies the high-pollen-hoarding syndrome. "I'd gotten stuck," Page recalls. Then in 2003, he read Amdam's Ph.D. thesis. A Norwegian graduate student, she had requested that Page be involved in her thesis defense.

CREDITS (TOP TO BOTTOM): CHARLES J. KAZILEK; MARY JANE WEST-EBERHARD

Bee lines. Differences between a honey bee strain that collected nectar (*right*) and one that stored pollen (*left*) helped researchers understand the division of labor in bees.

Amdam focused on a yolk protein called vitellogenin in the blood of worker bees. In solitary bees, vitellogenin is produced during the reproductive phase of the life cycle: Blood levels increase before egg-laying, when the bee starts foraging for pollen to provision the nest. Then they decline and remain low as it forages for nectar (see diagram). Amdam had established that nursing worker bees in a social hive produce vitellogenin and convert it into supplementary food for the hive's brood, and she proposed that the protein also regulates the timing and nature of foraging workers' behavior, through an inhibitory influence on juvenile hormone.

Among Page's bees, the high-pollen-hoarding workers tended to start foraging at an earlier age than the strains that had a preference for nectar. Amdam "had a biochemical explanation" that might possibly explain not just hoarding but other behavioral differences, says Page: "I thought, here it is, here is my entry point." Page suggested that his bees might provide a test of her thesis.

Amdam, who moved to the University of California, Davis, in 2004, collected bees from Page's colonies and tested their blood for levels of various reproductive factors, including vitellogenin. She found that young adults from the high-pollen-hoarding strains had higher levels of vitellogenin than those from the low-pollen-hoarding strains. These findings linked pollen-foraging behavior in sterile worker bees to a protein produced only during the "maternal" or reproductive phase of solitary bees. Amdam and Page proposed that the reproductive cycle of solitary bees became decoupled during evolution to produce a template for the foragers' division of labor in social bees. They called this idea the reproductive ground plan.

A close look at the workers' ovaries indicated that Amdam and Page had come up with molecular support for West-Eberhard's ovarian ground plan. High-pollen-hoarding bees tended to have twice as many egg-forming filaments—which correspond to a larger, more easily activated ovary—and were more likely to develop eggs if the colony lost its queen, Amdam and colleagues reported in 2006. Another study of 500 wild-type bees showed that those with more egg-forming filaments tended to be like the high-pollen-hoarding bees.

In 2007, the researchers cemented vitellogenin's connection to multiple aspects of

honey bee society when they used RNA interference to shut down the vitellogenin gene in wild-type bees. The workers began to forage at an even younger age, they preferred nectar, and they lived shorter lives.

The work "provided a link between reproductive potential and pollen foraging," says Christina Grozinger, who studies behavioral genomics at Pennsylvania State University, University Park. "The West-Eberhard ground plan provided a novel framework for interpreting data related to division of labor in social insects, while the Amdam-Page ground plan provided specific hypotheses which would be tested with molecular tools in honey bees."

Page, Amdam, and their colleagues have since converged on two genes that may explain both ovary size and behavior, which

in Australia, led by Benjamin Oldroyd and Madeleine Beekman, looked for correlations in ovary activation and division of labor in a strain of "anarchistic" bees in which some workers lay eggs even in the presence of a queen. They found that anarchistic workers foraged later in life, and some never make that transition. But they saw no correlation with active ovaries and pollen foraging, as the reproductive ground plan would have predicted, they reported 4 March 2008 in *PLoS Biology*.

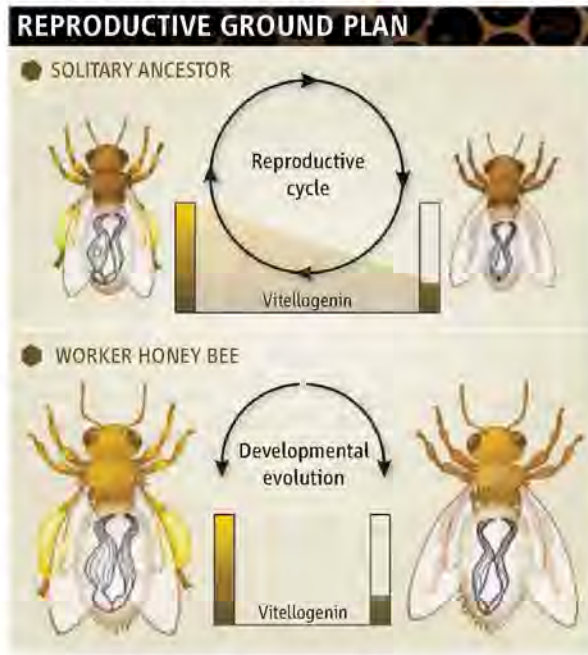
Other researchers are not yet convinced that the hypothesis is well-grounded. Ryszard Maleszka of the Australian National University in Canberra even calls the experimental evidence sketchy. "It is not 100% clear if the published experiments are conclusive or just correlations supported by statistics," he says. Moreover, "it's important to sort out to what extent environmental factors contribute to worker behavior and ovary size."

But Amdam, who moved to ASU in 2005, is not fazed by the criticism. ASU graduate student Adam Dolezal has evidence, reported in the May issue of *Animal Behaviour*, that a reproductive ground plan can influence the division of labor in the California harvester ant, *Pogonomyrmex californicus*. And Amdam's team has found that the honey bee's vitellogenin protein has a flexible region that could enable it to serve a regulatory role.

Seeking to establish causality between ovary size and worker behavior, the group has developed a way to transplant ovaries from one worker to another—say, one that comes from a high-pollen-hoarding strain to a bee from a low-pollen-hoarding strain, or between bees of different ages or behavioral states. In pilot studies, "we see the behavioral changes we expect, which is very exciting. We can now look at how the ovary is talking to the bees," says Amdam.

Amdam acknowledges that they have yet to show that the reproductive ground plan hypothesis can be applied broadly to other social insects. But she and others are pleased with their progress. "They keep going," says Bert Hölldobler, an entomologist at ASU, "and are getting more and more support for this concept."

—ELIZABETH PENNISI



Division of labor. Early in its life, a solitary bee's ovaries expand with the production and uptake of vitellogenin, encouraging pollen hoarding (*top*). After the egg develops, those levels drop, ovaries regress, and nectar feeding takes over. In worker honey bees, components of the solitary bee's life cycle are decoupled (*bottom*); high vitellogenin and larger ovaries lead to pollen hoarding, whereas low vitellogenin and smaller ovaries result in bees that are more interested in nectar.

they reported 2 April in *PLoS ONE*. One, *PDK1*, codes for a protein that may influence the number of egg-forming filaments produced during development, as well as food-related behavior in the adult bee; the other, *HR46*, is a nuclear hormone receptor that may determine ovary size in workers.

Building a case

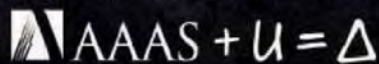
Not all the data fully support the reproductive ground plan hypothesis, however. In 2008, a team from the University of Sydney

An aerial photograph of a village in a dry, orange-brown landscape. The village is composed of numerous small, dark, rectangular structures, likely huts or houses, clustered together. A winding river or stream flows through the landscape, cutting through the orange-brown terrain. The overall scene is arid and desolate.

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LETTERS

edited by Jennifer Sills

Defending Freedom in Iran's Universities

ON 15 JUNE 2009, RIOT POLICE AND MILITIA ATTACKED THE UNIVERSITY of Tehran student dormitory, causing extensive damage, injuring 150 students, and killing at least one. This was just the latest example of brutal repression by the governments in power as a response to civil rights activism at Iranian universities.

In the first prominent example of this repression, three students were shot dead in December 1953 after protests against the visit by then-vice president Nixon to Iran (this event occurred after the CIA-backed coup in August of that year, which overthrew a democratically elected government and reinstated the Shah). Similar repression occurred at Iranian universities in 1978. In 1999, after a peaceful demonstration protesting the banning of a pro-reform newspaper, several students were sentenced to death for provoking civil unrest (the sentence was commuted to 15 years imprisonment following domestic and international outcry). The latest attack on Tehran University dormitory was followed by attacks on other universities such as Esfahan and Shiraz; arrested students remain in custody and information about those who were wounded or detained is unavailable. After about 4 months, Iranian authorities failed to identify and arrest the perpetrators of the attacks, leaving the issues in a haze of ambiguity. Recently, they expelled many students from Tehran Dormitory.

Over the past 4 years, Iran's universities have grown increasingly unhappy with the rigid academic as well as social restrictions established by the fundamentalists who run the government. Iran's univer-

sity movement remains largely frustrated due to lack of leadership, and lack of support from the universities themselves (most senior officials of which are appointed by the government).

Iranian student and academic movements have been pessimistic as to the support they might hope for from Western colleagues. Western universities and international scholarly societies should grasp the opportunity to rebuild trust with their Iranian colleagues by expressing their solidarity and support for Iran's universities and condemning the government's violence against them. However, nothing is more important in the days and weeks ahead than for Western governments to refuse to recognize conservative and fundamentalist Ahmadinejad as the next president of Iran. If Western governments recognize him, as they did with the Shah, it will be a major setback to the current civil rights movement and lead to the further isolation of Iran's scientists and academics. If the West shakes Ahmadinejad's hand now, it will be decades before Iran's scientists and students will be able to freely shake the hands of their counterparts in the West. After overwhelming pressure on universities in the last few months, hundreds of students recently staged an anti-government demonstration at several universities in Tehran. This shows that the freedom movement of Iranian universities is still alive.

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Restructure Conflicting Grant Incentives

ROALD HOFFMANN'S SUGGESTION TO SHIFT graduate students from grants to fellowships as a means of improving the quality of American science and science education ("Reshuffling graduate training," J. Mervis, *News Focus*, 31 July, p. 528) attempts to address the growing imbalance between the teaching and research obligations of academic science departments. Under the current system—in which students spend 2 years as teaching assistants in exchange for funding—faculty members pressure students to maxi-

mize their time on research, which conflicts with proper fulfillment of their teaching duties. Teaching assistants become de facto tuition-subsidized research assistants. With no alternative funds for teaching through grants, teaching support suffers, and the burden of quality instruction shifts onto already marginalized and overworked non-tenure track and adjunct faculty. Furthermore, when these graduate students become faculty, they often adopt the same attitude of favoring research over teaching, continuing the cycle of degrading science education.

The solution is to remove the incentives that lead faculty to shift departmental re-

sources away from teaching. Faculty requesting grants should be required to demonstrate how their entire research agenda can be achieved using only grant-funded graduate students. Faculty research programs requiring subsidization by teaching assistants would be compelled to re-budget to include such costs before receiving funding. Additionally, funding agencies should create positive incentives to value teaching through fellowship programs that fund teaching as part of the graduate training process. SADREDIN CYRUS MOOSAVI

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Stay Tuned for Multimedia Browsing

A. H. RENEAR AND C. L. PALMER ("STRATEGIC reading, ontologies, and the future of scientific publishing," Review, 14 August, p. 828) discuss the future of scientific publication. They show how coevolution of technology and working needs has led scientists to move from printed versions of articles toward diversified, digitized inputs that can be easily accessed, increasing the amount of information processed per day.

I predict that there will be a further paradigm shift. In the future, scientists will acquire more information more quickly by taking advantage of the higher amount of informative data and the higher perceptual impact that image and video communication have relative to written text (1). For this to happen, it will be necessary to develop algorithms, interfaces, and virtual environments that can translate video into accessible and searchable information. Effective video abstracting will pose a challenge in scientific video-based communication (2). Effective multimedia searches will allow increased information flow among scientists and a new practice of collecting scientific information, "browsing" not only through words but also through images and sounds.

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The Antidote to Bias in Research

THE MOST COMMONLY DISCUSSED SHIELD against bias in scientific practice is disclosure of financial conflicts of interest (COI). Such disclosure is necessary because the public wants it. Yet, a growing cadre of scientists question the value of disclosure policies (1, 2). These dissenters note that (i) such policies may actually increase biased behavior among some persons (3); (ii) judging scientists' credibility by their associations is tantamount to McCarthyism (4); (iii) financial interests are neither the sole nor necessarily the most compelling motives for COIs (5–7); and (iv) judging credibility of scientific conclusions based on characteristics of the scientist offering them is antithetical to the essence of science, which should rely on data and deductive reasoning alone (8). I add to this list that disclosure does

nothing to buttress the validity of the scientific information and conclusions produced. Given this, how can we ensure the validity of scientific information and conclusions in the face of the potentially biasing influences such as personal predilection, financial interests, philosophical leanings, and the search for personal aggrandizement? The answer lies in the methods of science itself.

Publicly sharing data offers one exemplary countermeasure. When data are public, no one need take analyses on faith. Anyone with the skills can conduct their own analyses, draw their own conclusions, and share those conclusions with others. This is more constructive than simply casting doubt on the analyses' integrity because of the analyst's affiliations.

The movement toward open data has begun. NIH, *Science*, the *Nature* journals, and other journals (9) all have policies encouraging or mandating it. Still, compliance with data sharing is challenging. Although *Nature Genetics* (NG) requires authors to publicly deposit data sets when publishing microarray gene expression studies, a recent study (10) found enough data to reproduce published work for only 2 of 18 papers analyzed. For the remainder, data were either not found or incomplete, or original analysis descriptions were insufficiently detailed to permit attempts

to replicate them. In another study, refusal to share data despite policies requiring sharing was nearly ubiquitous among authors publishing in Public Library of Science journals (11). This indicates that effective implementation of data sharing policies requires resources to support implementation and monitoring.

We must recognize that bias exists and exercise our creativity more and sanctimony less in seeking ways to minimize it. To paraphrase Adam Smith (12), with its focus on methods and processes, science itself is the antidote to the poison of bias in research.

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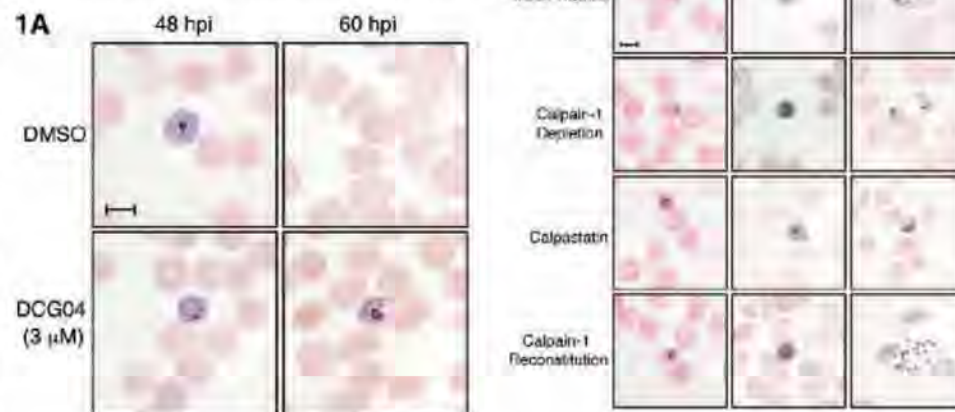
CORRECTIONS AND CLARIFICATIONS

Education Forum: "Revising the AP Biology curriculum" by W. B. Wood (25 September, p. 1627). The Web site address in reference 1 was incorrect. The correct address is <http://apcentral.collegeboard.com/apbiocFDRAFT>. The link has been corrected in the HTML version online.

Special Section on Carbon Capture and Sequestration: News: "Round and round: A guide to the carbon cycle" by D. Normile (25 September, p. 1642). The section titled "Dark mysteries" should have stated that humans are pumping 7 gigatons of carbon, not carbon dioxide, into the atmosphere each year.

Reports: "Emulation of a quantum spin with a superconducting phase qubit" by M. Neeley *et al.* (7 August, p. 722). Reference 20 cited the wrong paper. The correct reference 20 is "P. J. Leek *et al.*, *Science* **318**, 1889 (2007)."

Reports: "Apicomplexan parasites co-opt host calpains to facilitate their escape from infected cells" by R. Chandramohanadas *et al.* (8 May, p. 794). The following micrographs were inadvertently duplicated: (i) left-hand images in Fig. 1A, (ii) top-center image in Fig. 2B, and (iii) the first two panels in rows 2 and 3 of Fig. 2B. Thus, these experiments have been repeated by independent investigators, yielding the same conclusions as those originally reported. Corrected versions of the relevant figure panels, derived from these independent experimental replicates, are provided here.



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11. C. J. Savage, A. J. Vickers, *PLoS ONE* **4**, e7078, 10.1371/journal.pone.0007078 (2009).
12. A. Smith, *An Inquiry into the Nature and Causes of the Wealth of Nations* (T. Nelson and Sons, London, 1895), p. 333.
13. I am a frequent paid consultant to, and have received grants and donations from, many for-profit, non-profit, and government agencies. I thank K. Grimes for his helpful comments on an early draft of this manuscript.

Focus on Enhancing Phytochemical Content

DRAWING DEFINITIVE CONCLUSIONS ABOUT the possible nutritional benefits of organic foods based on analyses of the published literature is premature for the simple reason that so few valid studies, particularly long-term trials, have been conducted to date. Neither those who advocate for the nutritional superiority of organic food nor those who believe there is no difference have indisputable evidence for their conclusions. Even when screens are applied to select published studies that appear to be statistically valid, a more comprehensive perspective will emerge only as the body of work continues to increase.

As K. Clancy *et al.* admit ("Organics: Evidence of nutritional superiority is weak," Letters, 7 August, p. 676), there are enough published studies to suggest that organic fruits in particular might have higher levels of phytochemical compounds than those grown conventionally. However, the underlying causes for this possible difference are yet not well understood.

Clancy *et al.* assert that "cultivar selection may be as important or more important than the production method." This is a simplistic assessment. A multitude of additional factors could influence phytochemical content in fruits and vegetables, including fruit size, stage of development, ripening level, disease and/or pest pressure, soil conditions, fertilization practices, irrigation, pesticide application, season, location, climatic conditions including light intensity, and post-harvest processing and storage (1).

Rather than asking whether organic crops are nutritionally superior, we should ask whether it is possible for growers to enhance the phytochemical content of their crops and, if so, how. Scientists who are studying the nutritional profiles of crops

grown under organic and conventional management are taking steps toward answering this question.

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2. Part of X.Z.'s graduate work at Kansas State University was funded by grants from the Organic Farming Research Foundation.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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PLANETARY SCIENCE

The Passions of Meteorite Seekers

Dante S. Lauretta

Study of celestial objects is one of the few ways that a person can observe nature untarnished by human activities. As such, it has great appeal for amateurs and professionals alike. Any casual observer will witness bright streaks of light that appear briefly across the night sky. These meteors usually glow for about a second and leave trails that can last for a few minutes. A smaller percentage of skywatchers will catch a bright fireball that outshines the full Moon. Only a select few are fortunate enough to observe such cosmic debris survive its passage through the atmosphere and land safely on the surface of Earth. These meteorites are fragments of asteroids, Mars, and the Moon that have journeyed for thousands or millions of years since being liberated from their parent body. They have inspired countless individuals throughout history and have had a profound influence on human understanding of our place in the universe.

In *The Fallen Sky*, Christopher Cokinos offers a personal, often deeply intimate, journey through the history of meteorite collecting. His journey is motivated by an inner turmoil stemming from unhappiness in his personal life, guilt over infidelity, and the subsequent end of his marriage. These themes appear throughout the book, and Cokinos's character studies are often a reflection of his own state of mind. However, instead of proving to be a distraction from the characters who are revealed in individual chapters, the intertwining of the author's personal life with those of his subjects enriches each story and provides depth of character that is rare in a work of science history. The reader is filled with empathy for the people described in the volume and is left with a deep appreciation for the "exuberants" who are driven to search for, collect, and study meteorites.

Early chapters tell the story of Ellis Hughes, an Oregon farmer who finds an astonishing mass of

celestial iron in the woods, only to lose it in a seemingly endless series of legal battles. In other chapters, a poor farming family in Kansas finds meteoritic treasure in the soil of their croplands and an embattled Navy admiral finds redemption in the recovery of the largest meteorite removed from the field. These stories are brought to life by Cokinos, a poet and writer who teaches English at Utah State University. He not only performed meticulous research but also journeyed to many of the historic locales, including the coast of Greenland, the site where the famous Cape York iron meteorites were recovered by Robert Peary. The author's guilt manifests itself when he reveals his remorse for the way that sacred meteorites were removed from their terrestrial resting places and put on display in the great natural history museums of the world or worries about the repercussions of stealing a few bits of trash from Greenland.

The book is a great resource for learning about the people behind many of the great

legends in meteoritics. Cokinos does a masterful job describing the exploits of Daniel Barringer, an entrepreneur and miner in the American West who refuted the scientific findings of the most renowned geologist of the day (Grove Karl Gilbert) and revealed the nature of impact cratering on Earth's surface. The story of Harvey Nininger is similarly well crafted. A biology professor in Kansas, Nininger had his life transformed by a shoot-

ing star and went on to found the modern field of meteoritics. His conflicts with the caretakers of Meteor Crater as well as his rejection by Kansas homesteaders during an attempt to recover the Norton County aubrite, which ultimately led to a

nervous breakdown, provide a grim foreshadowing of events from the author's own life.

The book's final section chronicles the 2003–2004 field season of the Antarctic Search for Meteorites (ANSMET). A collaborative effort among three government agencies (the National Science Foundation, the National Aeronautics and Space Administration, and the Smithsonian Institution), ANSMET has been a scientific bonanza for meteoritics. Its collection efforts over the past 30 years have recovered over 16,000 meteorite samples, including many unique sam-

ples such as the first felsic asteroidal crust (1). Cokinos participated in the program as part of the NSF's Antarctic Visiting Artists and Writers program.

Ultimately, the author's gnawing guilt, his exhaustion from his extensive wandering in search of sites important to the history of meteoritics, and the extreme desolation and harsh conditions of the Antarctic plateau overwhelmed Cokinos. He was evacuated from the field and treated for severe depression. The ANSMET team rallied around their comrade and provided their full support. This crescendo of emotion proved to be the final release that Cokinos needed to find peace with himself and his decisions in life. He had traveled to

The Fallen Sky

An Intimate History of Shooting Stars

by Christopher Cokinos

Tarcher (Penguin), New York, 2009. 528 pp. \$27.95, C\$35. ISBN 9781585427208.



Peary's iron "Tent." Both the current and previous (shown) Hall of Meteorites at the American Museum of Natural History have been centered on this 30.8-metric-ton specimen from Cape York, Greenland.

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the ends of Earth searching for answers to questions that drive meteorite lovers to abandon the security and comforts of a mundane life to recover fragments of the heavens. He finds happiness in new love and a new life. In the end, Cokinos proves, as he states, that "the fallen sky can reveal secrets not only of the Solar System but of our hearts."

Although generally scientifically sound, the book contains a few key science gaffes. In particular, the author's description of the system for assigning petrologic type to a meteorite is confused, and his discussion of the Precambrian includes an incorrect assertion that Earth's orbital period was longer in the distant past. However, *The Fallen Sky* is not a textbook on meteorites, and there are plenty of other excellent sources for someone looking to brush up on the basics of meteoritics (2, 3). Instead, Cokinos guides the reader along his search for the driving force behind the passions of meteorite scientists, collectors, and dealers that make the meteoritic community such a vibrant and contentious bunch. It is a journey well worth taking.

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Exasperated by Copyright

Pamela Samuelson

How is it possible that a young woman in Duluth, Minnesota, Jammie Thomas, could have been ordered to pay Capitol Records \$1.92 million for peer-to-peer file sharing of 24 songs? Copyright industries persuaded the U.S. Congress to let them get "statutory damages" in any amount between \$750 and \$150,000 per infringed work that the judge or jury deems "just." The jury's award was \$80,000 per song, just about half the total possible award. Thomas cannot possibly pay that amount, but the award stands as a frightening message to other file sharers.

In *Moral Panics and the Copyright Wars*, William Patry explains how copyright law has

gone so far astray from its historical mission of promoting the progress of science (i.e., knowledge) by providing just enough protection to allow authors to obtain some reward for the artistic and literary works they contribute to our culture. Major copyright industries successfully convinced Congress that they have "property rights" in copyrighted works, such as sound recordings, and any music fan who downloads or shares her favorite music is a "thief" or a "pirate."

Drawing upon sociological and psychological studies, Patry claims that the copyright industries in general and the recording industry in particular have been inducing "moral panics" that have fueled the copyright wars against file sharers, among others. Moral panics, he explains, are "a reaction by a group of people based on the false or exaggerated perception that some cultural behavior or group, frequently a minority group or a subculture, is dangerously deviant and poses a menace to society." By exaggerating the risks of disastrous losses—such as the alleged billions of dollars in harm to the U.S. economy caused by file sharing—and engaging the press to report on them, the copyright industries have furthered a political agenda for obtaining ever-stronger and ever-longer copyright protection and maximizing criminal and civil liability for infringement.

Peer-to-peer filesharing is only the latest of numerous moral panics that copyright industries have induced in Congress and other policy venues in the past several decades. Especially well told here is the story of Hollywood's war against the Betamax machine in the 1970s and 1980s. Major movie studios were in the process of developing a new at-home movie-viewing format when Sony introduced its Betamax machine. Universal City Studios, among others, recognized that consumers might well prefer the Betamax to their new machine because the Betamax had time-shift copying and commercial-skipping features, neither of which the studios found acceptable. To stop this new competitor, Universal and Disney sued Sony for contributory copyright infringement.

The Motion Picture Association of America also waged war against the Betamax in Congress. The ever-colorful Jack Valenti asserted that the motion picture industry was "going to bleed and bleed and hemorrhage" unless Congress protected it "from the savagery and the ravages of this machine. He went on: "I say to you that the VCR is to the American film producer and the American public

as the Boston Strangler is to the woman home alone." While trying to persuade Congress to prohibit the Betamax from being distributed in the United States, Valenti also engaged in Japan bashing. It was, he said, "a piece of sardonic irony that ... while the Japanese are unable to duplicate the American films by a flank assault, they can destroy it by this video cassette recorder." Valenti's fears notwithstanding, the VCR was probably the best thing that ever happened to the motion picture industry. First VCR tapes and later DVDs became major profit centers for the film companies.

Some of the stories that Patry tells in the book have been told by others, including Larry Lessig (1) and Jessica Litman (2). An advantage of Patry's account is that he has first-hand experience of the stories he tells. He served as copyright counsel for the House of Representatives Committee on the Judiciary and as a policy adviser to

the Register of Copyrights. He has previously written a multivolume treatise on copyright law (3) and a book on the fair use doctrine (4). A long-time copyright insider, he has mastery of the legislative and policy battles that have been fought over the past 40 years.

At present, Patry is senior copyright counsel to Google, Incorporated. The book's last chapter discusses the creative destruction of old business models caused by the Internet and other information technology innovations. Yet, anyone looking for the inside scoop on how Google has won some of its copyright battles or on the Google Book Search settlement will be sorely disappointed, for there are no Google stories in *Moral Panics*.

Patry is a lively critic of major copyright industry groups and of the copyright policymaking process. He forcefully argues that "copyright is a utilitarian government program—not a property or moral right." One comes away from the book, however, wishing that he had offered some sage advice or insights about how to avert these moral panics and copyright wars. If he has good ideas about what a better copyright law might look like, he neglected to include them.

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by William Patry

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CLIMATE CHANGE

Parallel Pursuit of Near-Term and Long-Term Climate Mitigation

Stacy C. Jackson

It is well accepted that reduction of carbon dioxide (CO_2) emissions is the lynchpin of any long-term climate stabilization strategy, because of the long lifetime of CO_2 in the atmosphere (1). However, a focus on CO_2 may prove ineffective in the near term without comparable attention to pollutants with shorter lifetimes (2).

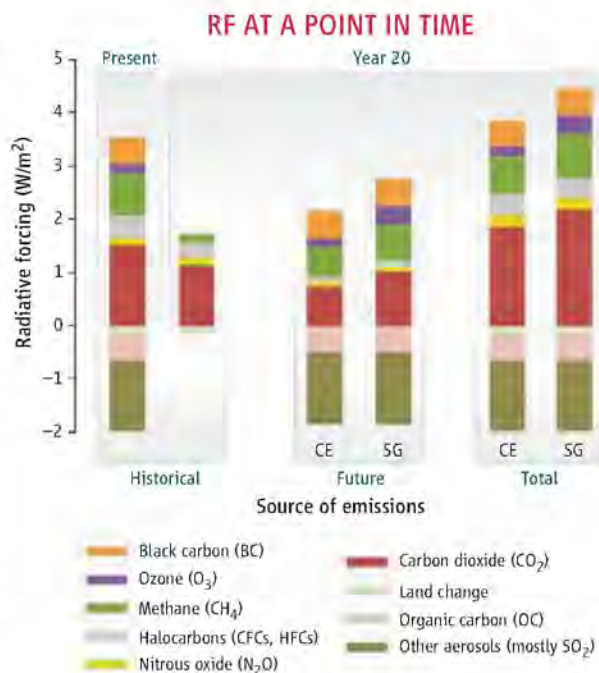
A growing body of evidence suggests that significant climate changes are no longer a distant prospect and that time spans on the order of decades are increasingly relevant (3). Observations over the past decade indicate that the climate is changing more quickly than projected by earlier Intergovernmental Panel on Climate Change (IPCC) Assessment Reports (4, 5), that climate impacts occur at lower surface temperatures than previously estimated (6), and that temperature changes will be greater during this century than had been previously projected (7). These faster-than-expected changes are occurring in the context of evidence of abrupt decadal change in the paleoclimate record (8), an evolving but incomplete understanding of "tipping points" and irreversible "points of no return" (9, 10), rapid approach to the level of radiative forcing (RF) (11) historically correlated with an ice-free planet (10), evidence that the rate of change of RF may influence the climate response (12), and modeling difficulty in replicating past abrupt climate changes (13).

Policy must evolve and incorporate the emerging sci-

ence in order to be effective. There is a growing need to create a two-pronged framework capable of not only mitigating long-term climate change but also managing the magnitude and rate of change of near-term RF.

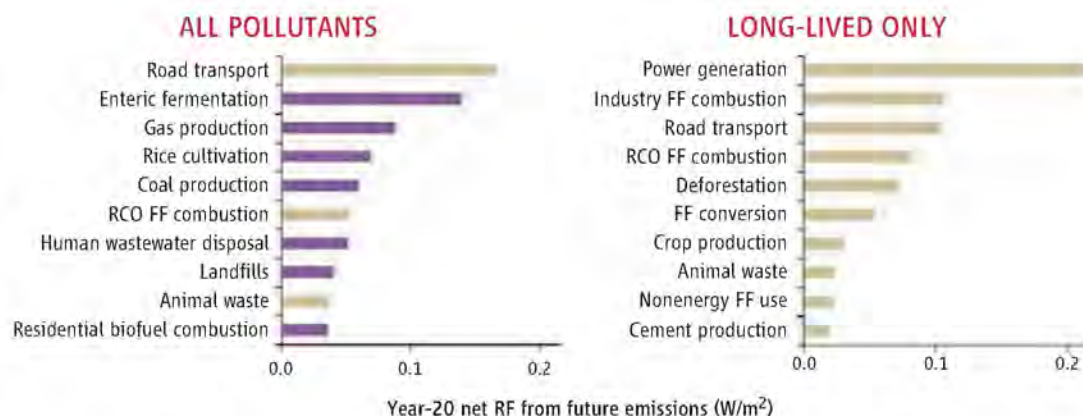
Mitigation of near-term climate change

Two separate treaties are needed to address the unique climate roles of long-lived versus medium- and short-lived pollutants.



Global radiative forcing. The leftmost bar shows RF attributable to historical human emissions (1750–2000). The next bar represents historical RF that would remain after 20 years of atmospheric decay, if one assumes zero additional human emissions in years 1 to 20. The next two bars represent RF in year 20 resulting from human emissions (beginning at 0 RF and incorporating atmospheric decay). Two scenarios are depicted: Emissions remain constant at year 2000 levels (CE), or emissions grow steadily at current rates (SG) (25). The rightmost columns show total RF experienced in year 20 (historical + future emissions) for the CE and SG scenarios (27). Ozone generated from atmospheric methane is included under methane. Land change includes physical changes in planetary reflectivity (albedo) and evapotranspiration caused by changes in surface vegetation cover. Emissions from land change are included under each pollutant (e.g., CO_2 includes deforestation).

involves different pollutants and source activities than mitigation of long-term climate change. Positive RF resulting from the next 20 years of human activity (see the top chart, bars 3 and 4) will exceed positive RF remaining, after decay, from historical human activity (bar 2). Short-lived pollutants (black carbon and tropospheric ozone) and medium-lived pollutants (methane) account for more than half (57 to 60%) of the positive RF generated in years 1 to 20. These findings



Top 10 global sources of year 20 net RF. Excludes historical emissions. Analysis combines results from the top chart (third bar, CE scenario) with source activity data from (28) and (32). SG scenario is omitted (insufficient data on activity-level growth rates). Long-lived pollutants (CO_2 , N_2O) have only positive RF; pollutants that are not long-lived have both positive (BC, O_3) and negative RF (OC, SO_2). Hence, a source may show higher or lower RF on the left versus right [e.g., power generation emits both warming (long-lived CO_2) and cooling (short-lived SO_2) pollutants, which offset at this time scale]. Halocarbons are excluded because activity data are not available for gases addressed by the Montreal Protocol. FF, fossil fuel; RCO, residential, commercial, and other. Data reflect uncertainty because of incomplete measurement and reporting infrastructure for non- CO_2 pollutants.

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complement prior studies that highlight the importance of short- and medium-lived pollutants (14–17).

The top 10 pollutant-generating activities contributing to net RF (positive RF minus negative RF) in year 20 are shown in the bottom chart, page 526, which takes into account the emission of multiple pollutants from each source activity (18). The seven sources that appear only on the left side (purple bars) would be overlooked by mitigation strategies focusing exclusively on long-lived pollutants.

The distinctly different sources of near-term and long-term RF lend themselves to the aforementioned two-pronged mitigation approach. This decoupling is convenient for policy design and implementation; whereas the importance of long-term climate stabilization is clear, the perceived urgency of near-term mitigation will evolve with our knowledge of the climate system. Additionally, optimal near-term mitigation strategies will reflect decadal oscillations (19), seasonal and regional variations (20, 21), and evolving knowledge of aerosol-climate effects (22, 23) and methane-atmosphere interactions (22)—considerations unique to the near term.

Thus, short- and medium-lived sources (black carbon, tropospheric ozone, and methane) must be regulated separately and dynamically. The long-term mitigation treaty should focus exclusively on steady reduction of long-lived pollutants. A separate treaty for short- and medium-lived sources should include standards that evolve based on periodic recommendations of an independent international scientific panel. The framework of “best available control technology” (strict) and “lowest achievable emissions rate” (stricter) from the U.S. Clean Air Act (24) can be used as a model.

Such a two-pronged institutional framework would reflect the evolving scientific understanding of near-term climate change, the scientific certainty around long-term climate change, and the opportunity to separately adjust the pace of near-term and long-term mitigation efforts.

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CLIMATE CHANGE

Fixing a Critical Climate Accounting Error

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Rules for applying the Kyoto Protocol and national cap-and-trade laws contain a major, but fixable, carbon accounting flaw in assessing bioenergy.

The accounting now used for assessing compliance with carbon limits in the Kyoto Protocol and in climate legislation contains a far-reaching but fixable flaw that will severely undermine greenhouse gas reduction goals (1). It does not count CO₂ emitted from tailpipes and smokestacks when bioenergy is being used, but it also does

not count changes in emissions from land use when biomass for energy is harvested or grown. This accounting erroneously treats all bioenergy as carbon neutral regardless of the source of the biomass, which may cause large differences in net emissions. For example, the clearing of long-established forests to burn wood or to grow energy crops is counted as a 100% reduction in energy emissions despite causing large releases of carbon.

Several recent studies estimate that this error, applied globally, would create strong incentives to clear land as carbon caps tighten. One study (2) estimated that a global CO₂ target of 450 ppm under this accounting would cause bioenergy crops to expand to displace virtually all the world's natural forests and savannahs by 2065, releasing up to 37 gigatons (Gt) of CO₂ per year (compa-

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rable to total human CO₂ emissions today). Another study predicts that, based solely on economic considerations, bioenergy could displace 59% of the world's natural forest cover and release an additional 9 Gt of CO₂ per year to achieve a 50% "cut" in greenhouse gases by 2050 (3). The reason: When bioenergy from any biomass is counted as carbon neutral, economics favor large-scale land conversion for bioenergy regardless of the actual net emissions (4).

The potential of bioenergy to reduce greenhouse gas emissions inherently depends on the source of the biomass and its net land-use effects. Replacing fossil fuels with bioenergy does not by itself reduce carbon emissions, because the CO₂ released by tailpipes and smokestacks is roughly the same per unit of energy regardless of the source (1, 5). Emissions from producing and/or refining biofuels also typically exceed those for petroleum (1, 6). Bioenergy therefore reduces greenhouse emissions only if the growth and harvesting of the biomass for energy captures carbon above and beyond what would be sequestered anyway and thereby offsets emissions from energy use. This additional carbon may result from land management changes that increase plant uptake or from the use of biomass that would otherwise decompose rapidly. Assessing such carbon gains requires the same accounting principles used to assign credits for other land-based carbon offsets.

For example, if unproductive land supports fast-growing grasses for bioenergy, or if forestry improvements increase tree growth rates, the additional carbon absorbed offsets emissions when burned for energy. Energy use of manure or crop and timber residues may also capture "additional" carbon. However, harvesting existing forests for electricity adds net carbon to the air. That remains true even if limited harvest rates leave the carbon stocks of regrowing forests unchanged, because those stocks would otherwise increase and contribute to the terrestrial carbon sink (1). If bioenergy crops displace forest or grassland, the carbon released from soils and vegetation, plus lost future sequestration, generates carbon debt, which counts against the carbon the crops absorb (7, 8).

The Intergovernmental Panel on Climate Change (IPCC) has long realized that bioenergy's greenhouse effects vary by source of biomass and land-use effects. It also recognizes that when forests or other plants are harvested for bioenergy, the resulting carbon release must be counted either as land-use emissions or energy emissions but not both.

To avoid double-counting, the IPCC assigns the CO₂ to the land-use accounts and exempts bioenergy emissions from energy accounts (5). Yet it warns, because "fossil fuel substitution is already 'rewarded'" by this exemption, "to avoid underreporting . . . any changes in biomass stocks on lands . . . resulting from the production of biofuels would need to be included in the accounts" (9).

This symmetrical approach works for the reporting under the United Nations Framework Convention on Climate Change (UNFCCC) because virtually all countries report emissions from both land and energy use. For example, if forests are cleared in Southeast Asia to produce palm biodiesel burned in Europe, Europe can exclude the tailpipe emissions as Asia reports the large net carbon release as land-use emissions.

However, exempting emissions from bioenergy use is improper for greenhouse gas regulations if land-use emissions are not included. The Kyoto Protocol caps the energy emissions of developed countries. But the protocol applies no limits to land use or any other emissions from developing countries, and special crediting rules for "forest management" allow developed countries to cancel out their own land-use emissions as well (1, 10). Thus, maintaining the exemption for CO₂ emitted by bioenergy use under the protocol (11) wrongly treats bioenergy from all biomass sources as carbon neutral, even if the source involves clearing forests for electricity in Europe or converting them to biodiesel crops in Asia.

This accounting error has carried over into the European Union's cap-and-trade law and the climate bill passed by the U.S. House of Representatives (1, 12, 13). Both regulate emissions from energy but not land use and then erroneously exempt CO₂ emitted from bioenergy use. In theory, the accounting system would work if caps covered all land-use emissions and sinks. However, this approach is both technically and politically challenging as it is extremely hard to measure all land-use emissions or to distinguish human and natural causes of many emissions (e.g., fires).

The straightforward solution is to fix the accounting of bioenergy. That means tracing the actual flows of carbon and counting emissions from tailpipes and smokestacks whether from fossil energy or bioenergy. Instead of an assumption that all biomass offsets energy emissions, biomass should receive credit to the extent that its use results in additional carbon from enhanced plant growth or from the use of residues or biowastes. Under any crediting system, credits must reflect net changes in carbon stocks, emissions of non-CO₂ greenhouse gases, and leakage emissions resulting from

changes in land-use activities to replace crops or timber diverted to bioenergy (1).

Separately, Europe and the United States have established legal requirements for minimum use of biofuels, which assess greenhouse gas consequences based on life-cycle analyses that reflect some land-use effects (1, 14). Such assessments vary widely in comprehensiveness, but none considers biofuels free from land-based emissions. Yet the carbon cap accounting ignores land-use emissions altogether, creating its own large, perverse incentives.

Bioenergy can provide much energy and help meet greenhouse caps, but correct accounting must provide the right incentives.

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Supporting Online Material

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PHYSICS

Glimpsing the Weak Magnetic Field of Light

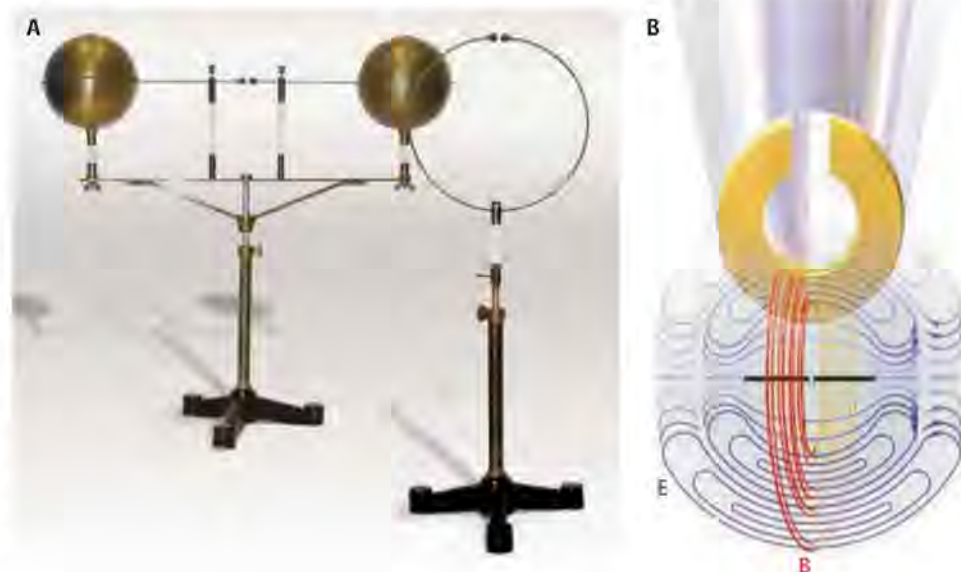
Harald Giessen¹ and Ralf Vogelgesang²

Since the work of James Clerk Maxwell and Heinrich Hertz, we have known that light is an electromagnetic wave. An intricate mechanism generates magnetic fields around the electric fields, and vice versa. In the optical-wavelength range, experimental studies have been limited to probing only the electric-field components. On page 550 of this issue, Burresi *et al.* (1) report direct measurements of the magnetic-field components of light obtained with a nanostructured metallic probe at the tip of a sharp glass fiber.

The instrument used by Burresi *et al.* can be viewed as a variant of the scanning tunneling microscope (2). Rather than imaging atoms on the surface, scanning near-field optical microscopy (SNOM) (3, 4) collects light from an object in the near field—that is, at a distance less than the wavelength of light λ . Thus, its resolution is not limited by the classical Abbe diffraction limit (roughly about $0.5 \lambda/\text{NA}$, where NA is the numerical aperture), which for infrared light is on the order of 500 nm.

SNOM allowed measurement of the local electric-field components of light, and hence the nanoscale optical features of surface plasmons, quantum dots, and individual molecules could be mapped. In the original setups, tapered fibers with subwavelength metallic holes at the end were used as probes. Subsequently, opaque tips allowed even higher resolution down to a few nanometers in so-called apertureless-scattering SNOM variants (5–8). More sophisticated variants of SNOM enabled researchers to determine the phase and the polarization of all three spatial vector components of the electric-field components of light (9). In the latter case, a linear polarizer in the setup allowed mapping of the three-dimensional character of the electric-field orientation.

The greater difficulty in determining the corresponding magnetic-field components of light arises from the weakness of this field relative to the electric field. The origin of the difference can be understood in a simplified picture with the Lorentz force, which describes the effects of magnetic and electric fields of light on moving charges. These charges could be the electrons in atoms or in solid-state nanostructures.



Divide and conquer. (A) Heinrich Hertz used this emitter (left) and receiver (right) to detect the magnetic component of electromagnetic waves. The spark inside the gap of the receiver, the first split-ring setup, was especially strong when the ring was aligned with respect to the magnetic field. (B) The magnetic field **B** of optical waves (red lines) can be detected with an interferometer that reads out the scattered light from a scanning near-field optical microscope with a metallic split-ring resonator at the tip of a glass fiber. The lines of the electric field **E** are shown in blue.

The ratio of the magnetic contribution to the electric counterpart scales as the ratio of the velocity of the charges v to the speed of light c . This ratio is the fine-structure constant α of atomic physics. For atoms, α is $1/137$; in solid-state systems, v is roughly the Fermi velocity and v/c is about $1/300$. The magnetic susceptibility χ_m scales as $(v/c)^2$, which makes magnetism weaker than its electric counterpart by four orders of magnitude (10). This difference in strength is the key reason why physicists have long ignored magnetism at optical frequencies, despite having been detected by Hertz for radio waves, where the wavelengths are centimeters to meters (see the figure, panel A). The circumference of the receiver scales roughly with the wavelength. In Hertz's case, the wavelength was about 3 m.

However, in a material that has structural features on a scale much smaller than λ , called a metamaterial, things are different (11). The magnetic moments in a metallic split-ring resonator (a ring with a notch in it) can be much greater than in atoms and conventional solids. The reason is that the magnetic flux is given by the product of current and area, and optical split-ring resonators can easily cover

An instrument has been fabricated that can detect the weak magnetic field of infrared light.

an area of 100 nm by 100 nm, or six orders of magnitude greater than the square of the Bohr radius in atoms.

Burresi *et al.* fabricated a metallic split-ring resonator at the tip of a glass fiber, which serves as a near-field optical probe (see the figure, panel B). The asymmetry created by the gap in the split ring causes the magnetic field to interact strongly with the nanostructure. This interaction couples the light into the structure and creates a measurable light signal at the other end of the fiber. Burresi *et al.* subsequently mixed this signal with reference light from their laser and extracted the amplitude and phase of the measured magnetic-field component at the fiber tip.

As an initial demonstration, Burresi *et al.* analyzed the magnetic field above an optical waveguide made from silicon nitride and showed convincingly that the detected magnetic-field signal is exactly 90° out of phase with the electric-field signal. When they replaced the split ring with a continuous ring, the signal vanished completely.

This method has the potential to give us a complete tomography of the optical vector field. One might envision a suitable nanoscopic

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scatterer, along with polarized detection, to determine all six optical vector field components (three electrical and three magnetic components). This capability is especially important for the design of complex nanoscopic geometries and intricate materials. Measurements could be made in the vicinity of sophisticated nanoantennas, in chiral (12) and multipolar metamaterials (13), in uniaxial and bianisotropic structures, as well as in multiferroics and high-temperature superconductors. Furthermore, spins in solids that are also associated with a magnetic moment could be assessed and controlled in the appropriate spectral region. One could send light into the SNOM fiber and convert it into a magnetic-field component at

the tip with the split ring. Also, magnetic dipole transitions in quantum emitters might show enhanced interaction with such a probe.

The electric- and magnetic-field components are intertwined with each other via the material properties and are described by the complex frequency-dependent permittivities and permeabilities. When the electric and magnetic optical responses can be measured independently in the vicinity of materials, we can obtain information about the complex local material properties. This capability may pave the way toward completely new effects, such as optically induced magnetism. We can only speculate as to how this concept might find application, such as in the read-write heads of

ultrahigh-density magnetic storage devices.

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VIROLOGY

A New Virus for Old Diseases?

John M. Coffin¹ and Jonathan P. Stoye²

There is little consensus in the medical community on whether chronic fatigue syndrome is a distinct disease. As its name implies, the condition is characterized by debilitating fatigue persisting for many years, and it affects as much as 1% of the world's population. Although chronic inflammation is often found in these patients, no infectious or toxic agent has been clearly implicated in this disease, which is diagnosed largely by excluding other conditions that cause similar symptoms (1). On page 585 of this issue, Lombardi *et al.* (2) describe the detection of xenotropic murine leukemia virus-related virus (XMRV) in about two-thirds of patients diagnosed with chronic fatigue syndrome. Both laboratory and epidemiological studies are now needed to determine whether this virus has a causative role, not only in this disease, but perhaps in others as well.

Chronic fatigue syndrome is not the first human disease to which XMRV has been linked. The virus first was described about 3 years ago in a few prostate cancer patients (3), and recently detected in nearly a quarter of all prostate cancer biopsies (4). It has been isolated from both prostate cancer and chronic fatigue syndrome patients, and is similar to a group of endogenous murine leukemia viruses (MLVs) found in the genomes of inbred and related wild mice. Although a half century of studies on MLVs and other gammaretroviruses have led to important dis-

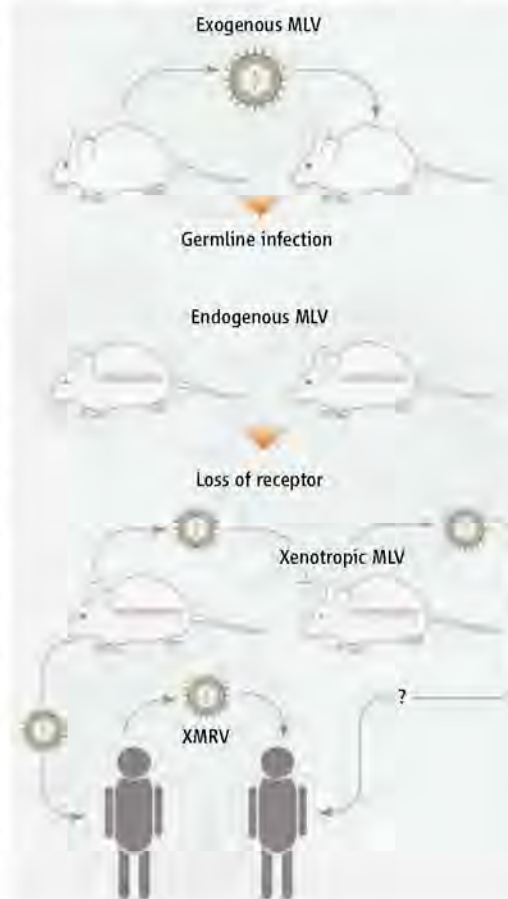
coveries on which much of our current understanding of cancer rests, there has been no clear evidence demonstrating human infection with gammaretroviruses, or associating these agents with any human disease.

Endogenous viruses, such as xenotropic MLV, arise when retroviruses infect germline

A retrovirus associated with cancer is linked to chronic fatigue syndrome.

cells. The integrated viral DNA, or provirus, is passed on to offspring as part of the host genome (see the figure). Endogenous proviruses form a large part of the genetic complement of modern mammals—about 8% of the human genome, for example. Xenotropic proviruses first entered the mouse germ line about a million years ago, but cannot infect cells of the mice that carry them because of a mutation in the cellular receptor for the virus presumed to have arisen after viral entry into the germ line. The propensity of xenotropic MLVs to infect rapidly dividing human cells has made them common contaminants in cultured cells, particularly in certain human tumor cell lines (5).

There is more than 90% DNA sequence identity between XMRV and xenotropic MLV, and their biological properties are virtually indistinguishable (6–9), leaving little doubt that the former is derived from the latter by one or more cross-species transmission events. There are several lines of evidence that transmission happened in the outside world and was not a laboratory contaminant. One is that XMRVs from disparate locations and from both chronic fatigue syn-



Path to human infection. Although xenotropic murine leukemia virus (MLV)—derived from exogenous MLVs that became established as proviruses in the mouse germ line—can no longer infect mice, it can infect humans, apparently leading to one or more cross-species infection events to become XMRV.

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drome and prostate cancer patients are nearly identical: The viral genomes differ by only a few nucleotides, whereas there are hundreds of sequence differences between XMRVs and xenotropic murine leukemia proviruses of laboratory mice. Other evidence includes the presence of XMRV and high amounts of antibodies to XMRV and other MLVs in chronic fatigue syndrome and prostate cancer patients.

There is still much that we do not understand. Whether the virus plays a causative role in either chronic fatigue syndrome or prostate cancer is unknown. For example, XMRV infection might, coincidentally, be more frequent in the same geographical region as that with a cluster of patients with chronic fatigue syndrome. And individuals with either disease might be more readily infected due to immune activation. XMRV might prefer to grow in rapidly dividing prostate cancer cells (10), and the presence of rapidly dividing cells in either condition might make infection more readily detectable. We do not know how the virus is transmitted, and the suggestion, based on indirect evidence, that there is sexual transmission (8) is premature. Given that infectious virus is present in plasma and in blood cells, blood-borne transmission is a possibility. Furthermore, we do not know

the prevalence or distribution of this virus in either human or animal populations, and animal models for infection and pathogenesis are badly needed.

Two characteristics of XMRV are particularly noteworthy. One is the near genetic identity of isolates from different diseases and from individuals in different parts of the United States. The two most distantly related genomes sequenced to date differ by fewer than 30 out of about 8000 nucleotides. Thus, all of the XMRV isolates are more similar to each other than are the genomes isolated from any one individual infected with the human immunodeficiency virus. In this respect, XMRV more closely resembles human T cell lymphotropic viruses (HTLVs) isolated from the same geographic region (11). As in the case with HTLV, the lack of diversity implies that XMRV recently descended from a common ancestor, and that the number of replication cycles within one infected individual is limited.

Another notable feature of XMRV is that the frequency of infection in nondiseased controls is remarkably high—about 4% in both normal individuals from the same geographic region as infected patients with chronic fatigue syndrome, and in nonmalignant prostates. If these figures are borne out in larger studies, it would

mean that perhaps 10 million people in the United States and hundreds of millions worldwide are infected with a virus whose pathogenic potential for humans is still unknown. However, it is clear that closely related viruses cause a variety of major diseases, including cancer, in many other mammals. Further study may reveal XMRV as a cause of more than one well-known “old” disease, with potentially important implications for diagnosis, prevention, and therapy.

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PLANETARY SCIENCE

A Lunar Waterworld

Paul G. Lucey

Since the first sample return missions of the 1960s, lunar scientists have operated under the presumption that the Moon is entirely dry. Three papers in this week's issue challenge that notion: Infrared spectroscopic measurements of the lunar surface from spacecraft provide unambiguous evidence for the presence of hydroxyl (OH) or water (1–3).

Early inspection of the lunar samples returned by the Apollo missions revealed none of the water-bearing primary minerals that are common in Earth rocks (4); instead, all the rocks examined were composed entirely of anhydrous minerals. Some Moon rocks also contain primary igneous metallic iron, which is extremely susceptible to alteration by water. No such alteration was found. Even in the case of coarse-grained plutonic rocks (which form from the cooling of mol-

ten rock below the surface), where volatiles in the magma chambers would have had ample opportunity to react with minerals, no hydrous phases were observed. This perceived dryness of the Moon stands in stark contrast to the abundant water found at Earth's surface and throughout its crust.

Recent reports of a few parts per million water detected in lunar glasses and phosphates with more sensitive methods suggest that the deep lunar interior may contain much more water than previously assumed (5, 6). However, the spatial pattern of OH and other volatiles in volcanic glasses suggests a diffusion profile (5), perhaps established while these erupting glasses lost water in flight through the lunar vacuum following eruption. Calculations of the original water contents suggest water abundances near 0.1% by weight, similar to those of terrestrial mid-ocean ridge basalts, indicating that the deep Moon may be as rich in water as is the deep Earth.

The new spacecraft measurements shift the focus of lunar water from the interior to

Space-based spectroscopic measurements provide strong evidence for water on the surface of the Moon.

the surface of the Moon. Infrared spectroscopic measurements from three spacecraft have unambiguously detected absorptions near 3 μm on the lunar surface that are almost certainly due to hydroxyl or water, or both (1–3). Three micrometers is the position of the fundamental vibrational absorption of the OH group and so is an extraordinarily sensitive indicator of the presence of water (or hydroxyl). Preliminary modeling by Sunshine *et al.* suggests a few tenths of a percent by weight water in the optical surface.

All three experiments show that the water-related absorption increases toward the lunar poles, although this observation does not necessarily mean that the concentration of a water-bearing phase increases toward the poles. Infill of the absorption feature by emitted thermal radiation will cause the depth of the apparent absorption to be temperature dependent. However, preliminary modeling by Pieters *et al.* takes this effect into account, yet the authors still observe a residual poleward increase in absorption depth, suggest-

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ing that the abundance of the host of the water or hydroxyl also increases with proximity to the lunar poles.

Pieters *et al.* also report local spatial variation in the strength of the 3- μm absorption associated with geologic features, especially areas rich in plagioclase feldspar, and suggest that these variations may be indicative of the deep water detected by Saal *et al.* (5). Sunshine *et al.* report that the spectral feature varies in strength with time, and suggest that the water is in a process of rapid and dynamic migration across the lunar surface.

These reports of lunar surface water coincide with intense interest in water at the poles of the Moon. Because the Moon's rotation axis has only a 1.5° tilt with respect to its orbit around the Sun, many polar craters are permanently shaded and should be at very low temperatures (some models suggest as low as 40 K) (7–9). These cold surfaces should trap volatile material migrating near the polar regions. That notion has given rise to several space-based remote-sensing experiments aimed at detecting possible polar water (or other volatiles). On 9 October of this year, the LCROSS (Lunar Crater Observation and Sensing Satellite) mission will try to detect water by deliber-

ately crashing a large spacecraft into a polar cold trap, thereby lofting lunar soil into sunlight for inspection by other spacecraft and ground-based observatories (10).

The results of the present studies lend credence to the lunar polar water hypothesis by providing a proven source of water on the surface of the Moon. Several extralunar sources are possible for polar water (11), but the latest observations provide a proximate source to supply a polar water deposit.

What is the ultimate source of the water detected? Important continuous sources of water include reduction of lunar divalent iron in minerals to metallic iron by solar-wind hydrogen, producing water, and liberation of water from the impact of interplanetary dust and small meteoroids. Water may also be implanted episodically by large comets or asteroids, but these require a mechanism for retention of water (probably in the form of chemically reacted hydroxyl).

These sources must be reconciled with the lack of any obvious evidence of any alteration of sampled lunar materials by water.



Lunar water. Infrared spectroscopic observations of the Moon (1–3) provide evidence revealing that it may not be as dry as assumed.

There may be much “wetter” regions to be discovered far from the sites that have been sampled to date. It is also possible that rare water-bearing minerals previously observed in lunar samples (12), but argued to be terrestrial contamination (13), might be indigenous. Perhaps the most valuable result of these new observations is that they prompt a critical reexamination of the notion that the Moon is dry. It is not.

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CHEMISTRY

Carbenes in Action

Martin Albrecht

Many chemists are drawn to molecules that behave like feral cats, reacting rapidly and often violently and resisting attempts to control them. But are they really as unpredictable as judged at first glance? Can we master their activity and perhaps even put it to use? Two reports in this issue describe the domestication of such molecules. On page 556, Bertrand and co-workers disclose the unexpected stabilization of “abnormal carbenes” (1), and on page 559, Lavallo and Grubbs use carbenes in new organometallic reactions (2). Both studies open up new and exciting opportunities in synthesis.

Carbenes have a divalent carbon that has

only six valence electrons (see the first figure, panel A). This electron deficiency violates Lewis' octet rule, rendering carbenes highly reactive (3). Carbenes were long considered too reactive for isolation and study. In recent years, cooling to just a few kelvin has been used to slow down chemical reactions and thereby stabilize carbenes (4). However, at these low temperatures, reactions may take place that are not relevant under normal conditions (just imagine riding a bicycle at extremely slow speed).

In the late 1980s, Bertrand and co-workers developed another method to stabilize carbenes. They combined electronic stabilization and steric protection to synthesize the first free carbenes stable at room temperature (5). Introducing two nitrogen atoms next to the carbene carbon further enhanced the stability of these carbenes and made them resis-

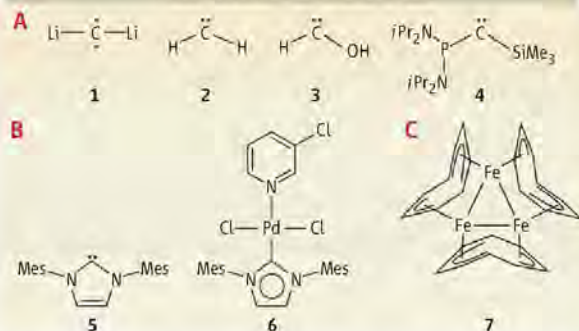
Chemists are taming a highly reactive class of molecules for use in catalysis and synthesis.

tant toward air and moisture (6). Once sufficiently tamed, these carbenes—particularly N-heterocyclic ones—rapidly became very useful for synthesis, especially in homogeneous catalysis and as ligands for transition metals (see the first figure, panel B) (7).

Catalysis with carbenes has largely concentrated on constructing chemical bonds from and to carbon, an area where carbenes are much more efficient than—although often not fundamentally different from—the more ubiquitous phosphines. Lavallo and Grubbs take a different direction, using N-heterocyclic carbenes as ligands for the formation of iron-iron bonds. The reaction is catalytic, leading to the formation of the starlike molecule (see the first figure, panel C). This rare case of a catalytic organometallic transformation challenges the paradigm that metal-carbene bond making and breaking are one-way processes.

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NORMAL CARBENES



Catalysis with carbenes. (A) The highly reactive carbenes **1** to **3** are unstable at room temperature. Carbene **4** was the first carbene shown to be stable at room temperature. (B) The N-heterocyclic carbene **5** is stable at room temperature. In **6**, it is bound to a highly catalytically active palladium center (Me, methyl; Mes = mesityl = 2,4,6-trimethylphenyl). (C) Lavallo and Grubbs prepared the starlike tri-iron compound **7** by using a saturated analog of carbene **5** as the catalyst for iron-iron bond formation.

The turnover numbers observed by Lavallo and Grubbs—despite being low—imply that the carbene is used multiple times as a temporary ligand. It remains to be seen whether reversible metal-carbene bond formation is general or limited to iron and related metals.

Such bond making between iron centers may help to construct electronically active systems. For example, variation of the carbene catalyst may enable the fabrication of iron chains. Because iron undergoes swift oxidation and reduction processes—thus acting as a source and a receptor for electrons, respectively—such “molecular wires” may be useful for molecular electronics.

Modifications in the skeleton of N-heterocyclic carbenes have a pronounced effect on their electron donor ability. Such modifications have been used to create “abnormal carbenes” (**8**), in which only one nitrogen is located next to the carbene carbon (see the second figure, panel A). This situation reduces their stability while increasing their ability to donate electron density to a coordinated metal center, thereby changing the reactivity of the metal center (**9**). Because of the low heteroatom stabilization, free abnormal carbenes have been considered to be unstable under ambient conditions, representing a considerably wilder cat than do normal N-heterocyclic carbenes.

Bertrand and co-workers (*1*) now describe an elegant strategy for creating abnormal carbenes that are stable at room temperature and can be crystallized, allowing for a detailed structural analysis. Building on their earlier success in stabilizing typically reactive carbenes (*10*), they introduced aryl substituents on all nuclei but the carbene carbon. The substituents assist in balanc-

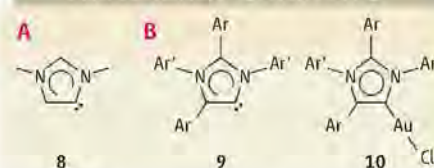
ing the charge density in the heterocycle. Steric shielding may be less relevant, because carbon dioxide or metal centers such as Au(I) readily bind to the carbene carbon (see the second figure, panel B).

This work may help to determine whether abnormal carbenes contain a divalent carbon (and are thus true carbenes), or whether they are zwitterions containing a carbanion. Sophisticated calculations (*1*) suggest that abnormal N-heterocyclic carbenes have electronic configurations very similar to those of their normal carbene equivalents. This close analogy, combined with the absence of any component in the crystal struc-

ture that could compensate a putative negative charge at the carbene carbon (*1*), tends to support the carbene notion. Further data will be essential, however, to clarify this aspect.

The availability of stable abnormal carbene ligands also offers new opportunities for synthesis, not least by providing access to a plethora of new highly reactive metal complexes for catalysis. Normal carbenes rapidly became a key tool for organometallic chemistry and organic synthesis once they were available as stable free ligands some 20 years

ABNORMAL CARBENES



Challenging the norm. (A) The unstable compound **8** is an abnormal N-heterocyclic carbene. (B) The abnormal carbene **9** reported by Bertrand and co-workers (*1*) is the first of its class to be stable at room temperature. The authors have also synthesized its gold(I) complex **10** (Ar = aryl).

ago. Given the unique impact of abnormal carbenes on the reactivity of transition metals, the accessibility of free abnormal carbenes may become another cornerstone in this field, and it will be exciting to witness developments in these directions.

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MOLECULAR BIOLOGY

Rejoice—RNAi for Yeast

Danesh Moazed

An RNA silencing mechanism with broad regulatory capability has finally been demonstrated in one of the most widely used model organisms.

The budding yeast *Saccharomyces cerevisiae* has been instrumental in discovering molecular mechanisms of fundamental cellular processes in eukaryotes, including cell cycle control, transcription, chromatin structure, and signal transduction. Missing from this picture has been RNA interference (RNAi), a major regulatory pathway in eukaryotic cells that silences gene expression through small interfering RNAs (siRNAs) that bind target sequences (*1–3*). On page 544 of this issue, Drinnenberg et al. (*4*) show that RNAi is

indeed present in some budding yeasts and can be restored in *S. cerevisiae*, opening exciting possibilities for studies focused on the biological functions and impact of RNAi in these organisms.

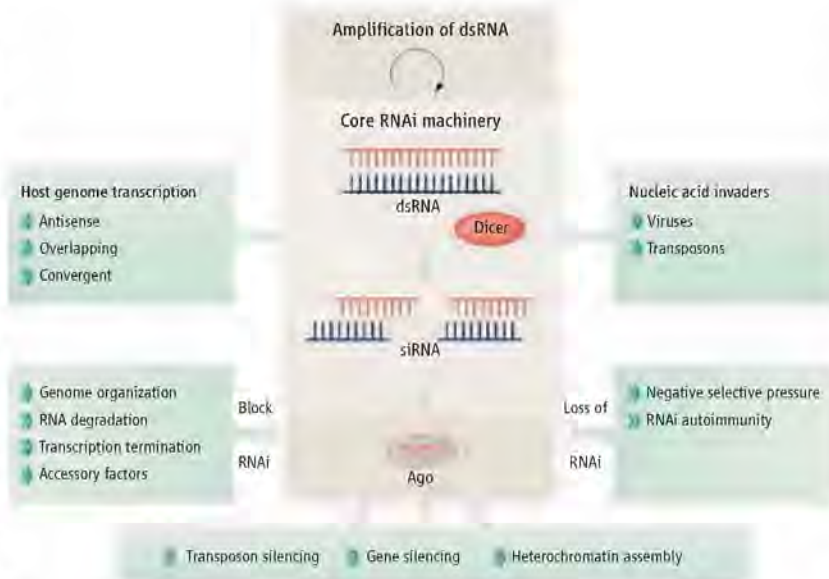
RNAi is an ancient mechanism present in plants, animals, and most fungi, but thought to have been lost during the evolution of budding yeasts. One of its major functions is defense against nucleic acids of invaders such as viruses, and against mobile segments of the host genome (transposons and retroelements), and could be considered a type of genetic immune system (*1, 2*). The mechanism is triggered by the processing of double-stranded RNA (dsRNA) into siRNAs about 23 nucleotides in size by Dicer, an enzyme of

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the ribonuclease III (RNase III) family (1) (see the figure). siRNAs are assembled onto an effector protein called Argonaute (Ago), which uses base-pairing interactions to target and inactivate cognate RNAs or to repress transcription. In some systems, RNAi is amplified by RNA-dependent RNA polymerases that synthesize dsRNA. Another branch of the pathway uses small RNAs (microRNAs) and has been estimated to modulate the expression of up to a third of human genes at the post-transcriptional level (5). In plants, ciliates, some animal cells, and the fission yeast *Schizosaccharomyces pombe*, RNAi promotes the assembly of silent chromatin structures (3). How could a pathway with such broad functions be absent in budding yeast?

To address this question, Drinnenberg *et al.* focused on budding yeasts whose genomes encode an Ago homolog. Unlike their close relative *S. cerevisiae*, the budding yeasts *Saccharomyces castellii*, *Kluyveromyces polysporus*, and *Candida albicans* (an opportunistic human pathogen) have Ago homologs. High-throughput sequencing of small RNA libraries from these yeasts revealed abundant small RNAs about 23 nucleotides in size, similar to those associated with RNAi in other systems (4). In *S. castellii*, most of the sequenced RNAs mapped to both genomic DNA strands or to transcripts that form a hairpin structure, suggesting that they originated from dsRNA precursors. Furthermore, the presence of small RNAs correlated with the presence of an enzymatic activity in yeast extracts that cleaves dsRNA into discretely sized small RNA.

Although no Dicer homologs were identified in these yeast genomes, Drinnenberg *et al.* discovered a noncanonical Dicer with proper RNA cleaving activity. However, this variant (Dcr1) is closely related to RNase III enzymes involved in RNA processing pathways that are independent of RNAi (6). Most Dicers contain two RNase III domains and a PAZ domain, which acts as a molecular ruler by fixing one end of dsRNA relative to RNase III active sites (7). Dcr1 is unusual in that it contains only one RNase III domain and, like the *S. pombe* Dicer (8),



The core of silence. Dicer and Ago form the core of RNAi mechanisms. RNA silencing may feed back on the pathways that generate siRNA to exert positive or negative effects. RNAi is also negatively regulated by factors that prevent dsRNA formation or modulate the activities of the core machinery. Inappropriate RNAi may target important cellular RNAs, resulting in RNAi autoimmunity. Negative selective pressure may have contributed to RNAi loss in some budding yeast species during evolution.

lacks a PAZ domain and must use an alternative measuring mechanism. Dcr1 is distinguished from Dicer by the presence of a binding domain for dsRNA, and the authors propose that it acts as a homodimer so that each RNase III domain cleaves one strand of the dsRNA precursor. Thus, Dcr1 proteins define a previously unrecognized family of Dicer enzymes.

The authors identified about 1000 loci in the *S. castellii* genome that generate siRNAs, but the abundance of transcripts targeted by these molecules generally did not change upon deletion of the genes encoding Dcr1 and Ago. This suggests that for most *S. castellii* loci, the expression of siRNAs is below the threshold required to associate with Ago and target RNAs. Nevertheless, the authors show that the RNAi pathway is capable of robust gene silencing. For example, they observed strong silencing of a reporter gene when siRNA was produced from a hairpin RNA corresponding to the reporter.

Remarkably, introduction of *S. castellii* genes encoding Dcr1 and Ago into *S. cerevisiae* reconstituted RNAi and efficiently mediated silencing of reporter genes (4). Furthermore, unlike *S. castellii*, *S. cerevisiae* harbors active transposons in its genome. Reconstituting RNAi in *S. cerevisiae* silenced the transposition of a Ty1 transposon. This silencing appears to result from the processing of overlapping transcripts into Ty1 siRNAs, which in turn mediate the destruction of Ty1 mRNA. Inspection of all

transcripts in *S. cerevisiae* revealed RNAs that were antisense to transposon elements. This suggests that a major role of RNAi in budding yeasts involves the silencing of transposons, but it is unclear whether other transcripts with the potential to form dsRNA exist and can be similarly silenced.

As proposed by Drinnenberg *et al.*, loss of RNAi may result from a lack of positive selection, such as occurs during evolutionary periods in which transposons are dormant. Another possibility is that like any potent destructive force, RNAi is under negative selective pressure. This is probably evident in the radiation of mechanisms that modulate RNAi

and restrict biogenesis of the dsRNA trigger (see the figure). For example, the evolution of gene arrangements that prevent formation of dsRNA, combined with RNA degradation mechanisms, curtail the ability to form dsRNA (9, 10). Restrictive mechanisms involving factors that limit siRNA production, such as siRNA ribonucleases, or proteins that control siRNA loading onto Ago, have also been observed (11, 12). In *S. pombe*, RNAi is limited to nascent heterochromatin-bound RNAs (3). And *S. cerevisiae* and some other budding yeast species appear to have gotten rid of RNAi altogether. Thus, RNAi is likely to have had a profound impact on the evolution of the eukaryotic genome and its potential to produce RNAs that can become double-stranded. It should now be possible to determine the price of reacquiring RNAi on *S. cerevisiae* fitness.

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Lab Experiments Are a Major Source of Knowledge in the Social Sciences

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Laboratory experiments are a widely used methodology for advancing causal knowledge in the physical and life sciences. With the exception of psychology, the adoption of laboratory experiments has been much slower in the social sciences, although during the past two decades the use of lab experiments has accelerated. Nonetheless, there remains considerable resistance among social scientists who argue that lab experiments lack "realism" and generalizability. In this article, we discuss the advantages and limitations of laboratory social science experiments by comparing them to research based on nonexperimental data and to field experiments. We argue that many recent objections against lab experiments are misguided and that even more lab experiments should be conducted.

The social sciences have generally been less willing to use laboratory experiments than the natural sciences, and empirical social science has traditionally been considered as largely nonexperimental, that is, based on observations collected in naturally occurring situations. The first lab experiments in economics were not conducted until the late 1940s. Fewer than 10 experimental papers per year were published before 1965, which grew to about 30 per year by 1975 (1, 2). Starting from this low level, experimentation in economics greatly increased in the mid-1980s. In three well-known economics journals—*American Economic Review*, *Econometrica*, and *Quarterly Journal of Economics*—the fraction of laboratory experimental papers in relation to all published papers was between 0.84% and 1.58% in the 1980s, between 3.06% and 3.32% in the 1990s, and between 3.8% and 4.15% between 2000 and 2008 (authors' calculations). The percentages were much higher in more specialized economics journals. The first specialty journal, *Experimental Economics*, was founded in 1998. A similar increase in lab experiments has taken place in other social sciences as well, for example, in political science (3).

Many social scientists are still reluctant to rely on laboratory evidence. Common objections are that student participant pools are unrepresentative and that sample sizes are small. There is also a widespread view that the lab produces unrealistic data, which lacks relevance for understanding the "real world." This notion has its basis in an implicit hierarchy in terms of generating relevant data, with field data being superior to lab data. We argue that this view, despite its intuitive appeal, has its basis in a misunderstanding of the nature of evidence in science and of the kind of data collected in the lab. We also

"Economists unfortunately ... cannot perform the controlled experiments of chemists or biologists because they cannot easily control other important factors. Like astronomers or meteorologists, they generally must be content largely to observe."

Samuelson and Nordhaus
Principles of Economics 12th ed.
McGraw-Hill New York 1985, p. 8

"Experimental economics is an 'exciting new development'."

Samuelson and Nordhaus
Principles of Economics 14th ed.
McGraw-Hill New York 1992, p. 5

argue that many of the objections against evidence from the lab suggest the wisdom of conducting more lab experiments, not fewer. Although most of our examples and topics are taken from economics, the methodological points we discuss can be applied to all social sciences.

The Lab Provides Controlled Variation

Controlled variation is the foundation of empirical scientific knowledge. The laboratory allows tight control of decision environments. As an illustration, consider a simple experiment, the gift-exchange game, which tests the theory that employment relationships are governed by a gift exchange; that is, that workers reciprocate fair wages with high effort. A positive relationship between wages and effort is the central assump-

tion of efficiency wage theories that have important implications for the functioning of labor markets and that can explain rigid wages and involuntary unemployment (4). Testing this class of theories with field data is notoriously difficult. For example, in firms, worker effort is not easily observed or measured, and workers are confronted with a mix of different incentives. This makes interpretation of different effort levels difficult. An observed variation in wages may not reflect generosity but may be due to firm size, self-selection of workers, or simply productivity differences. Even if a relationship between wages and effort is detected, it may not necessarily reflect a fair-wage-effort relationship; instead, it could reflect strategic considerations based on reputation and repeated interactions. In the laboratory, these factors can be varied in a controlled fashion. The experimenter observes effort and wages and can rule out confounding effects such as multiple incentives, selection, productivity differences, and repeated interactions.

The first experimental test of the existence of gift exchanges in the framework of a formal game-theoretic model was designed to mimic an employment relationship. Participants assumed the roles of workers and firms (5). Firms made (binding) wage offers, which workers could accept. If a worker accepted, he or she then had to choose a costly effort level. Labor contracts are generally incomplete contracts; that is, effort is not fully contractually enforceable. In the experiment, this is reflected by the fact that workers were free to choose any effort level above the contractually enforceable level. In this framework, it is possible to test the gift-exchange hypothesis against the self-interest assumption commonly made in economics: that a self-interested worker would always choose the lowest possible effort because effort is costly

and there is no punishment for minimal effort. Anticipating this, the firm has no incentive to pay an above-minimum wage, because self-interested workers work no harder if given a higher-than-minimum wage. Nevertheless, the results of numerous gift-exchange experiments in the lab revealed that higher wages induce workers to provide higher effort levels. The experiment is a good example of the many experiments that have challenged the assumption of a universally selfish and rational *Homo economicus*. Systematic lab evidence shows that people are boundedly rational and prone to behavior such as loss aversion, present bias, or judgment biases (6). Phenomena such as reciprocity or social approval, which have been largely neglected by mainstream economics, have been shown to be important in affecting

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economic outcomes in bargaining and market interactions (7, 8).

These experiments illustrate that the lab offers possibilities to control decision environments in ways that are hard to duplicate with the use of naturally occurring settings. In the laboratory, the experimenter knows and controls the material payoffs, the order in which the different parties can act, the information parties possess when they make choices, and whether the game is repeated or one shot. This control allows for the testing of precise predictions derived from game-theoretic models. Participants are randomly assigned, and decisions are rewarded. Payment ensures that participants take their decisions seriously. For example, if a firm pays a higher wage or a participant provides higher effort, costs are higher and final earnings are lower. In this sense, behavior in the laboratory is reliable and real: Participants in the lab are human beings who perceive their behavior as relevant, experience real emotions, and take decisions with real economic consequences (6, 9–12). Lab experiments can be used for testing theories and to study institutions at relatively low cost (9). This is particularly interesting for policy questions where the proposed program intervention has no counterpart in reality and where constructing the counterfactual states of interest may be done more easily in the lab than in the field. Moreover, whereas existing institutions are adopted endogenously, rendering causal inferences about their effects difficult, the lab allows exogenous changes in institutions. Lab experiments have turned out to be valuable in solving practical problems that arise in implementing matching markets (13), government regulation (14), or airport time-slot allocation (15). Economic engineering, a combination of theory and experiments, has improved design and functioning of many markets (16).

Lab or Field Is Not the Choice

Resistance concerning laboratory evidence often centers on an appeal to realism. This skepticism has recently manifested itself in a lively debate in economics about field versus lab experiments. Whereas some scholars have argued passionately in favor of laboratory experiments where controlled manipulations of conditions on carefully documented populations are performed (17–19), others have argued in favor of field experiments where conditions are more “realistic,” although perhaps less tightly controlled, and where more realism also implies greater relevance to policy (3, 20–22). These controversies appear throughout the social sciences (3, 23–26).

The casual reader may mistakenly interpret arguments about realism as an effective critique against the lab, potentially discouraging lab experimentation and slowing down the production of knowledge in economics and other social sciences. The issue of realism, however, is not a distinctive feature of lab versus field data. The real issue is determining the best way to isolate the causal effect of interest. To illustrate this point

and to structure the debate about field and lab data, we suggest the following simple model.

Consider an outcome of interest Y (e.g., effort supplied by a worker) and a list of determinants of Y , $(X_1, \dots, X_N)$. For specificity, suppose that

$$Y = f(X_1, \dots, X_N) \quad (1)$$

which is sometimes called an all-causes model (27, 28) because it captures all possible causes of Y in $(X_1, \dots, X_N)$. The causal effect of X_1 on Y is the effect of varying X_1 holding fixed $\tilde{X} = (X_2, \dots, X_N)$. In the pioneering field experiments (20), X_1 was the tax rate on wages. In the laboratory gift-exchange experiment, the values of X_1 are the different wage levels paid by firms. Unless f is separable in X_1 , so that

$$Y = \phi(X_1) + g(\tilde{X}) \quad (2)$$

the level of Y response to X_1 will depend on the level of $\tilde{X}$. Even in the separable case, unless $\phi(X_1)$ is a linear function of X_1 , the causal effect of X_1 depends on the level of X_1 and the size of the variation of X_1 . These problems appear in both field and lab experiments and in any estimation of the causal effect of X_1 .

Among the $\tilde{X}$ in the gift-exchange experiments described above are concrete details of market institutions such as the number of firms and workers, the order of moves, the choice set, payoff functions, information available, whether or not interactions are one shot, and whether or not they are anonymous. More generally, $\tilde{X}$ could be demographic characteristics of the participants, the level of observation of actions by third parties, individual preference parameters (e.g., morality, persistence, self-control, and social and peer influences), and other aspects of environments.

Many laboratory experiments like the gift-exchange experiment have provided evidence of gift exchange and social preferences in lab settings for certain values of $\tilde{X}$, usually, but not always, with use of populations of undergraduates and different bargaining and market institutions (7, 29). The relevance of these findings has been questioned in recent field experiments analyzing behavior in a population of sports-card traders in a “natural setting,” that is, for another set of conditions $\tilde{X}'$ including, for example, different institutional details, payoffs, and a different participant population (30). In this particular market, the evidence for social preferences is weaker. If one is interested in the effect of social preferences under a third condition ($\tilde{X}''$), neither the undergraduate nor the

sports-cards field study may identify the effect of interest. It is not obvious whether the lab $\tilde{X}$ or the field $\tilde{X}'$ is more informative for the third condition unless a more tightly specified economic model is postulated or a more precisely formulated policy problem is specified. Recent evidence suggests that empirical support for the existence of social preferences such as reciprocity or gift exchange is not a matter of lab or field but of the prevailing conditions such as the details of agent interactions (31–33). When the exact question being addressed and the population being studied are mirrored in an experiment, the information from it can be clear and informative. Otherwise, to transport experimental findings to new populations or new environments requires a model (34).

Field methods are able to obtain a universally defined causal effect only if the special functional form (Eq. 2) is specified and the response of Y to X_1 is linear. If this is the case, however, lab experiments are equally able to obtain accurate inferences about universal effects. Observing behavior in the field is in general not more interesting or informative than observing behavior in the lab. The general quest for running experiments in the field to obtain more realistic data is therefore misguided. In fact,

“While laboratory processes are simple in comparison to naturally occurring processes, they are real processes in the sense that real people participate for real and substantial profits and follow real rules in doing so. It is precisely because they are real that they are interesting.”

Charles R. Plott

Journal of Economic Literature
vol. 20, 1982, p. 1486

the key issue is what is the best way to isolate the effect of X_1 while holding constant $\tilde{X}$. No greater reality is conveyed by one set of $\tilde{X}$ than another unless the proposed use of an estimate as well as target populations and settings are carefully specified. The pioneering field experiments (20) defined the target population and the questions sought to be answered very precisely.

The usefulness of particular methods and data is ultimately a matter of the underlying research question. Lab experiments are very powerful whenever tight control of $\tilde{X}$ is essential. This applies in particular for testing (game theoretic) models and behavioral assumptions. The lab can also easily implement many different values of $\tilde{X}$, for example, the number of buyers or sellers in market experiments, and in this way explore the issue of robustness of an estimated effect. Tight control of $\tilde{X}$ also allows replicability of results, which is generally more difficult with field data. The field on the other hand offers a large range of variations in $\tilde{X}$, which are potentially relevant but hard to implement in the lab. This way field experiments can provide important complementary insights to lab findings, for example, in the area of development economics, or by addressing specific policy questions such as testing antipoverty programs that are targeted to, and need to be evaluated on, populations in poverty (20).

Other objections. In addition to the lack-of-realism critique, other objections concerning lab evidence have been put forward. Ironically, most objections raise questions that can be very well analyzed with lab experiments, suggesting the wisdom of conducting more lab experiments, not fewer. One common objection is that lab experiments with students do not produce representative evidence. For the purpose of testing theories, this is not a problem because most economic models derive predictions that are independent of assumptions concerning participant pools. On some aspects of behavior, however, students are not representative of the general population or a target population of interest, and we would agree that a richer variation in context, populations, and environments $\tilde{X}$ should be used in future lab experiments. In this vein, the gift-exchange game has been run on nonstudent samples (35), yielding results similar to those obtained by using samples of students. Other studies have shown the relevance of social preferences for CEOs (36), for professional financial traders (37, 38), or for the general population (39, 40).

It has also been noted that (i) stakes in experiments (money paid for decisions taken) are trivial, (ii) the number of participants or observations is too small, (iii) participants are inexperienced, (iv) Hawthorne effects may distort experiments, or (v) self-selection into experiments may bias results.

(i) Most of what we know about the level of stakes on outcomes is derived from controlled lab experiments. The effects of varying stake size are mixed and seem to depend on concrete experimental contexts (41). Reciprocity does not vanish if participants in the gift-exchange experiment reported above are paid an equivalent of three months of income (42). Even if stake effects are relevant, however, it is not obvious what the "right" level of stakes should be; that is, what are the right levels of X_1 and $\tilde{X}$? We would ask in reply, how often do people make

decisions involving monthly incomes, and how representative would such high-stake experiments be for the many decisions people make on a daily basis, which involve relatively small stakes? In any case, if one is seriously interested in how stakes affect behavior, one can run experiments with varying stake sizes.

(ii) The issue of sample size is a red herring. Effective methods have been developed for analyzing small sample experiments (43–45). Moreover, many experiments nowadays are run with samples of several hundred participants, sometimes with more than 1000 participants (46).

(iii) Experiments do not typically distinguish between experienced and inexperienced participants. It is an empirically interesting question how experience, learning, etc. affect behaviors. Failure to account for experience in some experiments is not an intrinsic weakness of the experimental method. In fact, it is common to run experiments with experienced participants and to study learning effects. Good examples are a study on ratchet effects and incentives with Chinese managers (47) and a study comparing the behavior of workers and students (48). In a recent field experiment on gift exchange, it was shown, for instance, that experienced donors, that is, donors who frequently donate for a particular charitable organization, reciprocally respond to gifts by donating more frequently (32).

(iv) Another concern often raised is scrutiny, that is, the possibility that participants in the lab behave differently because they perceive that they are observed. This is one version of a Hawthorne effect. [Parenthetically, reanalysis of the original Hawthorne data shows that no Hawthorne effect was present in the Hawthorne study (49).] It is a minor problem in many experiments, especially if the decision environment is interactive and "rich," such as in sequential bargaining or market experiments. Moreover, being observed is not an exclusive feature of the laboratory: Many decisions outside the lab are observed. Even on the Internet agents can be observed. In the lab, observers can be added to the experimental protocol. The lab allows the analyst to study the relevance of scrutiny by varying the degree of anonymity, for example, contrasting video experiments where participants are explicitly observed, with single anonymous (participant-participant anonymity) and double anonymous (full anonymity between participants and experimenter) procedures (50–52).

(v) Many scholars have expressed concerns about self-selection of particular participants into experiments. Self-selection is not necessarily a scourge. It can be a source of information on agent preferences (27, 28, 34). In the lab, one can collect detailed data on the backgrounds and personality traits of participants to control for selection or to explicitly study selection in a controlled way (53, 54). Selection is a feature of both field and social experiments (55) and is not a problem unique to lab experiments. Indeed, problems of noncompliance, attrition,

and randomization bias plague many field experiments (27, 56).

Exploiting Complementarities

Experiments can be productive in complementing the information obtained from other empirical methods. One can combine lab and field experiments to better understand the mechanisms observed in the field. For example, this can be done by eliciting preferences and relating these preferences to observed behavior in the field (57, 58). Another example of exploiting complementarities is the experimental validation of survey instruments (59). Whereas surveys can generate large and representative data sets that provide statistical power, experiments allow the elicitation of preferences and attitudes in a controlled and incentive-compatible way because participants have to make choices with real money at stake. Such evidence is particularly important in securing better understanding of preference heterogeneity (60–63). The evidence that people are different clashes sharply with the widely used "representative agent" model that assumes that agents are homogenous or can be represented as if they are homogenous. Accounting for heterogeneity in preference parameters enables macroeconomists to calibrate economic models in an empirically founded way (61).

We conclude by restating our argument. Causal knowledge requires controlled variation. In recent years, social scientists have hotly debated which form of controlled variation is most informative. This discussion is fruitful and will continue. In this context, it is important to acknowledge that empirical methods and data sources are complements, not substitutes. Field data, survey data, and experiments, both lab and field, as well as standard econometric methods, can all improve the state of knowledge in the social sciences. There is no hierarchy among these methods, and the issue of generalizability of results is universal to all of them.

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Smallest Algae Thrive As the Arctic Ocean Freshens

William K. W. Li,^{1*} Fiona A. McLaughlin,² Connie Lovejoy,³ Eddy C. Carmack²

As global climate changes, conditions will favor some organisms more than others; there will be ecological winners and losers. In the Arctic, rising air temperature, increasing precipitation, higher river flows, and declining snow cover have lead to large and rapid change in the upper ocean. Surface waters in the Canada Basin have also freshened in recent years because of increased sea ice meltwater and episodic input of large river runoff (1). The reduction of sea ice in summer, which is occurring more rapidly than forecasted (2), may affect phytoplankton production. As the ice edge retreats away from the continental shelf break, wind-driven upwelling of deep nutrient-rich waters can be expected to enhance shelf production (3). A greater open sunlit area and a longer growing season also combine to increase annual primary production (4); however, Arctic phytoplankton production appears to be limited by the supply of nitrogen and not cumulative irradiance (5). The constraints and requirements imposed by nutrients differ among phytoplankton types, so the response to change presumably differs.

Here, we show that, in the changing Arctic Ocean, the smallest phytoplankton cells thrive but larger cells languish. Although the time series of basinwide summer averages is short, the trend of a warmer and fresher upper ocean is evident (Fig. 1A) from a repeated survey of 23 stations (figs. S1 and S2). The density of deep water has remained about the same over this period, so the decreasing density of the upper ocean results in stronger stratification (Fig. 1A). Similarly, deep water nutrients have not changed, but upper ocean

nutrients have decreased (Fig. 1A). Picoplankton, being very small (<2 μm diameter), have a large surface-area-to-volume ratio that provides effective acquisition of nutrient solutes and photons, as well as hydrodynamic resistance to sinking. Predictably (6), these cells increased (Fig. 1B) in a regime of lower nitrate supply and greater hydrodynamic stability. Conversely, larger nanoplankton (2 to 20 μm) decreased (Fig. 1B). Upper ocean bacterioplankton increased at the same relative rate ($\sim 10\%$ year⁻¹) as picophytoplankton, but deep ocean bacterioplankton remain unchanged (Fig. 1C), suggesting that heterotrophic and photosynthetic changes are coupled in the picoplankton. A reduction in community average body size because of an increase in the abundance of individuals belonging to small-sized species may be a common response to global warming (7).

Total phytoplankton biomass, represented as the universal photosynthetic pigment chlorophyll *a*, remained unchanged (Fig. 1B). This biomass, alternately represented as the sum of diagnostic pigments (8), is largely ($\sim 85\%$) a complementary mix of cells containing either chlorophyll *b* (picoplanktonic green flagellates) or fucoxanthin (microplanktonic diatoms) (Fig. 1D). Prasinophytes, especially a genetically unique pan-Arctic cold-adapted ecotype of *Micromonas*, constitute a large proportion of picophytoplankton in these waters (9). Accepting a time-for-space substitution, the observed increase in picoplankton may thus be associated with a redistribution of pigment groups within the community observed across stations. A secular trend cannot be discerned without a much longer observational time series because of

inherent interannual variability. However, if current changes persist, an altered food web may be expected because community size structure is a strong determinant of ecosystem carbon flux. Picoplankton-based systems tend not to support large exports of biogenic carbon, either for extraction (e.g., harvest) or for sequestration (e.g., burial).

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Supporting Online Material

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Figs. S1 and S2
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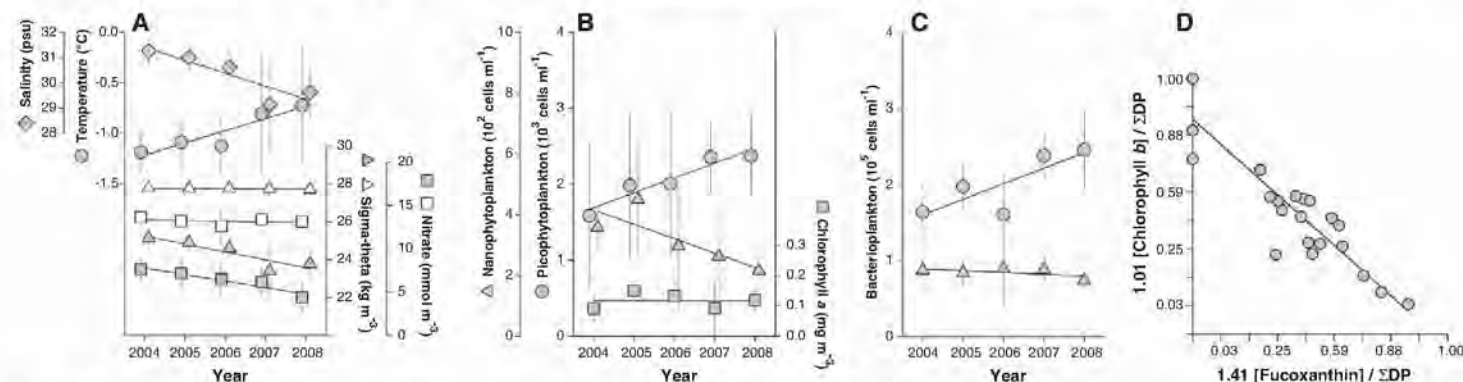


Fig. 1. Summer conditions in the Canada Basin. (A) Upper ocean (gray symbols) temperature ($P = 0.05$), salinity ($P = 0.0004$), density ($P = 0.001$), and nitrate ($P = 0.06$); deep ocean (open symbols) density ($P = 0.8$) and nitrate ($P = 0.9$). (B) Upper ocean picophytoplankton ($P = 0.01$), nanophytoplankton ($P = 0.09$), and chlorophyll *a* ($P = 1.0$). (C) Upper ocean (circles, $P = 0.09$) and deep ocean (triangles, $P = 0.3$) bacterioplankton. Error

bars are standard deviation of station averages (fig. S1); probability values test for significance of linear regression. (D) Proportion (p) of phytoplankton biomass (ΣDP is the sum of diagnostic pigments) represented by green flagellates (1.01Chl*b*) versus diatoms (1.41Fucoxanthin) from 2007 station survey shown on angular transformed scale (arcsin $p^{1/2}$) for normalization of platykurtic distribution, according to pigment scheme of Uitz et al. (8).

Intraspecific Polymorphism to Interspecific Divergence: Genetics of Pigmentation in *Drosophila*

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Genetic changes contributing to phenotypic differences within or between species have been identified for a handful of traits, but the relationship between alleles underlying intraspecific polymorphism and interspecific divergence is largely unknown. We found that noncoding changes in the *tan* gene, as well as changes linked to the *ebony* gene, contribute to pigmentation divergence between closely related *Drosophila* species. Moreover, we found that alleles linked to *tan* and *ebony* fixed in one *Drosophila* species also contribute to variation within another species, and that multiple genotypes underlie similar phenotypes even within the same population. These alleles appear to predate speciation, which suggests that standing genetic variation present in the common ancestor gave rise to both intraspecific polymorphism and interspecific divergence.

Similar phenotypes that vary within and between species may or may not be caused by similar genetic mechanisms. Quantitative trait mapping shows that loci contributing to polymorphism and divergence of a single character map to the same region of the genome approximately half of the time (table S1). These overlapping quantitative trait loci (QTLs) may or may not result from changes in the same genes, and most studies lack the power to distinguish between these possibilities. To determine whether the same genes (and potentially even the same alleles of these genes) contribute to phenotypic diversity within and between species, one must resolve intra- and interspecific QTLs to individual genes, localize functionally divergent sites within these genes, and then compare specific alleles within and between species.

***ebony* and *tan* QTLs contribute to pigmentation divergence.** To investigate the relationship between intraspecific polymorphism and interspecific divergence, we examined the genetic basis of pigmentation differences within and between a pair of closely related *Drosophila* species, *D. americana* and *D. novamexicana*. These two species are sister taxa within the *Drosophila virilis* species group that diverged about 300,000 to 500,000 years ago (1, 2) (Fig. 1A). *D. novamexicana* has a derived light yellow body color, whereas other members of this group (including *D. americana*) retain an ancestral dark brown body color (3) (Fig. 1B). In the laboratory, these species can mate and produce fertile offspring. Genetic mapping showed that a

region of the second chromosome containing the *ebony* gene contributes to pigmentation divergence between *D. novamexicana* and *D. americana* (4). This gene is required for pigmentation in *D. melanogaster* (5). Three other autosomal regions, as well as an unidentified region of the X chromosome, also contribute to pigmentation divergence, although none of these regions were linked to other pigmentation genes tested (i.e., *yellow*, *dopa-decarboxylase*, *optomotor blind*, and *bric-a-brac*).

Recently, the X-linked pigmentation gene *tan* was cloned in *D. melanogaster* (6). To test whether this gene might contribute to pigmentation differences between *D. americana* and *D. novamexicana*, we crossed *D. americana* females to *D. novamexicana* males, backcrossed F₁ hybrid females to *D. novamexicana* males, and scored 495 backcross progeny for body color (fig. S1). All of the lightest male offspring (*n* = 10) inherited the *D. novamexicana* allele of *tan*, whereas all of the darkest male offspring (*n* = 24) inherited the *D. americana* allele of this marker. These data show that sequences linked to *tan* contribute to pigmentation divergence (*P* = 8×10^{-9} ; Fisher's exact test). The previously described pigmentation QTL linked to *ebony* and the lack of a pigmentation QTL linked to *yellow* (4) were also reconfirmed in this population (*P* = 3×10^{-8} and 0.7, respectively; Fisher's exact test).

To determine the phenotypic effects of QTLs linked to *ebony* and *tan*, we created lines of *D. novamexicana* in which genomic regions containing these genes were replaced with orthologous sequences from *D. americana*. These genotypes were constructed by marker-assisted introgression, moving *ebony* and *tan* alleles from *D. americana* into *D. novamexicana*. F₁ hybrid females were backcrossed to *D. novamexicana*

males, and a single female inheriting the *D. americana tan* (or *ebony*) allele was randomly selected and backcrossed to *D. novamexicana* males again. This process was repeated for 10 generations (fig. S2), with females carrying the *D. americana ebony* or *tan* allele selected randomly in each generation without regard to pigmentation. Introgressed *D. americana* sequences linked to either *tan* or *ebony* darkened pigmentation relative to wild-type *D. novamexicana* (Fig. 2, A to C), with sequences linked to *ebony* (Fig. 2C) causing darker pigmentation than sequences linked to *tan* (Fig. 2B). Digital quantification of pigmentation showed that, when combined, the introgressed *tan* and *ebony* regions recapitulated 87% of the pigmentation difference between species (Fig. 2, A, D, and E).

***ebony* and *tan* affect pigmentation development.** Studies of pigmentation in *D. melanogaster* suggest that *ebony* and *tan* may themselves be responsible for these interspecific QTLs. Loss-of-function mutations in *ebony* darken pigmentation (5), whereas loss-of-function mutations in *tan* lighten it (7). Biochemically, Ebony catalyzes the conversion of dopamine into *N*-β-alanyl-dopamine (NBAD), which is a precursor for (yellow) sclerotin, and Tan catalyzes the reverse reaction, converting NBAD back into dopamine, which is a precursor for (brown) melanin [reviewed in (8)] (Fig. 3A). Ectopic expression of Ebony induces yellow pigmentation (9) (Fig. 3D), whereas ectopic expression of Tan induces brown pigmentation (6) (Fig. 3E). Ectopic ex-

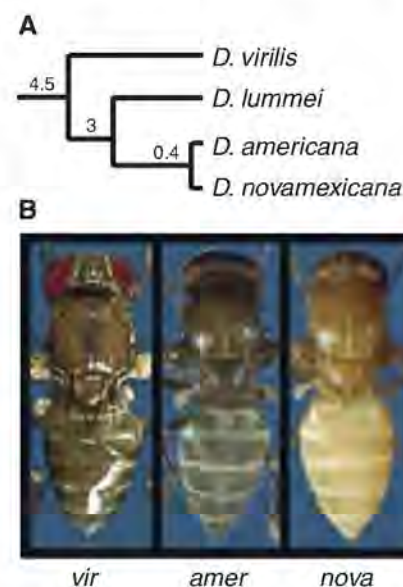


Fig. 1. *D. novamexicana* yellow body color is derived. (A) Phylogenetic relationships among members of the virilis phylad within the virilis group of *Drosophila* are shown with estimated divergence times (1) at each node (numbers denote millions of years ago). (B) Dorsal body pigmentation is shown for *D. virilis* (*vir*), *D. americana* (*amer*), and *D. novamexicana* (*nova*). *D. lummei* (not shown) has pigmentation similar to that of *D. virilis* and *D. americana* (3).

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pression of both proteins simultaneously results in pigmentation intermediate to that caused by ectopic expression of either protein alone (Fig. 3F), showing that the balance between Ebony and Tan enzymatic activity affects pigmentation. Genetic and biochemical pathways controlling pigment synthesis are highly conserved among insects (10), which suggests that the *D. americana* and *D. novamexicana* *tan* and *ebony* genes function similarly to their *D. melanogaster* orthologs. Consistent with this prediction, the Ebony protein is more abundant in epidermal cells of the yellowish *D. novamexicana* during late pupal stages than in the darker *D. americana* (4).

Noncoding changes in *tan* contribute to pigmentation divergence. The above results are consistent with changes in *ebony* and *tan* contributing to pigmentation divergence, but

they cannot be used to distinguish divergence affecting these genes from divergence affecting linked loci. This is particularly concerning for *ebony* because it is located in a part of the genome that is inverted between species (4, 11). Inversions effectively suppress recombination, precluding genetic dissection of the region. Nonetheless, differences in Ebony protein expression between *D. americana* and *D. novamexicana* (4) strongly suggest that this gene is involved in pigmentation divergence.

Unlike *ebony*, *tan* is in a freely recombining region of the genome. This allowed us to use fine-scale genetic mapping to separate the effects of *tan* from neighboring genes and to determine whether *tan* contributes to the altered pigmentation observed in the *tan* introgression line (Fig. 2B). A 2.7-kb region of *tan* was identified that contributes to pigmentation divergence

(fig. S3) and contains 57 single-nucleotide differences and 19 insertions or deletions (indels) (fig. S4). All of these changes affect noncoding sequences, and the region includes the entire first intron (fig. S3). Differences located 3' of this region must also affect pigmentation, however, because the recombinant fly inheriting *D. americana tan* sequence only in this region was not as dark as flies inheriting *D. americana* sequence for the full *tan* gene (fig. S3). Within *tan*, this 3' region includes many noncoding differences as well as two nonsynonymous differences that affect amino acids 190 and 267.

***tan* expression correlates with pigmentation differences.** Given the absence of coding changes in the 2.7-kb mapped region of *tan*, we expect that divergent sites in this region affect pigmentation by altering *tan* expression. Because of its darker pigmentation, we hypothesized that *D. americana* has higher levels of *tan* expression than *D. novamexicana*. In situ hybridization showed that *tan* is expressed throughout each dorsal abdominal segment ("tergite") in both species during the P14 and P15 pupal stages (12) when pigmentation develops (fig. S5, A and B). This expression pattern correlates with the distribution of pigments in adult *D. americana* and *D. novamexicana* tergites, and is distinct from the patterns of *tan* expression in *Drosophila* species with other pigment patterns (13). Differences in *tan* expression detected with in situ hybridization correlate with pigmentation divergence in these other species (13), yet we saw no obvious expression differences between *D. americana* and *D. novamexicana* during the same developmental stages with this technique (fig. S5, A and B).

To quantitatively compare levels of *tan* expression, we measured the relative abundance of *tan* transcripts in stage P14 and P15 pupae of each species with Pyrosequencing (14). We observed an average of 34% more *tan* transcripts in *D. americana* females than in *D. novamexicana* females ($n = 4$ samples, each containing six flies; $t = 3.7$, $P = 0.03$; t test) (fig. S5, C and D), consistent with the darker pigmentation of *D. americana*. To determine whether this expression difference results from cis-regulatory divergence of *tan*, we compared transcript abundance of *D. americana* and *D. novamexicana tan* alleles in F_1 hybrid females during the same pupal stages with Pyrosequencing (14). Surprisingly, no significant differences in allele-specific expression were observed ($n = 5$ samples, each containing six flies; $t = 0.72$, $P = 0.51$; t test) (fig. S5, E and F). Divergent expression levels may therefore be caused by differences in trans-regulatory factors and/or differences in the number of *tan*-expressing cells between species (15). The noncoding differences we identified by fine-scale genetic mapping may alter fine-scale temporal control (16) and/or posttranscriptional regulation (17) of *tan*. It is also possible that these noncoding differences may affect transcriptional regulation of a neighboring gene that is also

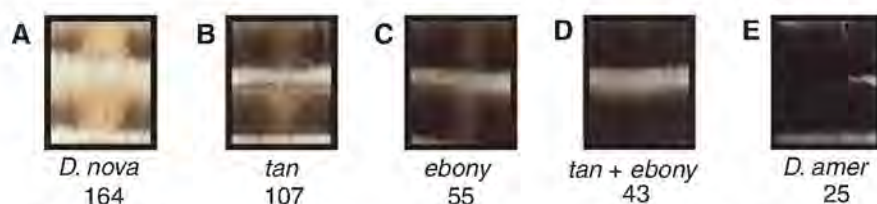


Fig. 2. QTLs linked to *tan* and *ebony* account for the majority of pigmentation divergence between species. Dorsal abdominal cuticle is shown from segments A4 and A5 of 7- to 10-day-old adult females. (A to C) Relative to *D. novamexicana* (A), introgression of alleles linked to *tan* (B) or *ebony* (C) led to darkened pigmentation. (D and E) Together, the introgressed regions produced even darker pigmentation (D), although these flies were not as dark as wild-type *D. americana* (E). Numbers indicate intensity of grayscale images, where 0 = black and 255 = white. Panels (B), (C), and (D) are all heterozygous for the introgressed region(s).

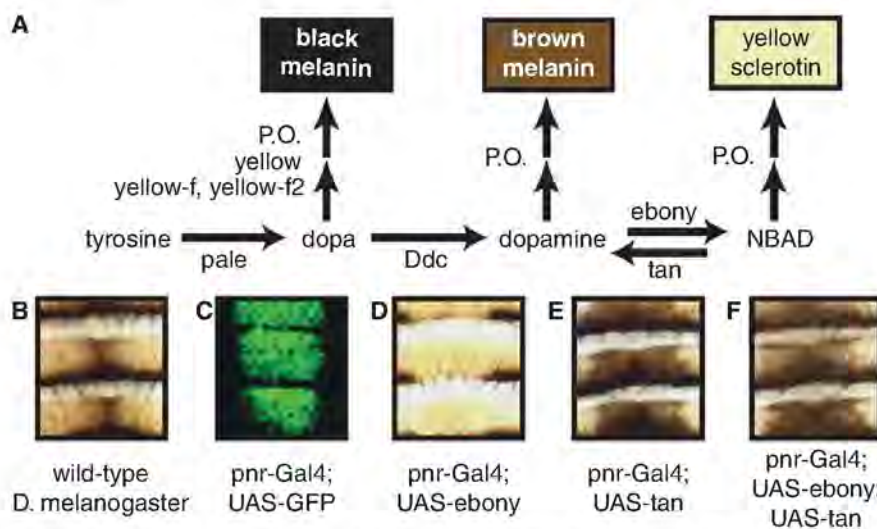


Fig. 3. Ebony and Tan have reciprocal effects on pigmentation development. (A) A simplified melanin biosynthesis pathway is shown. For a more complete pathway, see (6). The gene(s) controlling each enzymatic step are shown in italics. P.O. indicates genes encoding phenol oxidase proteins. Branches with two consecutive arrows include multiple enzymatic steps that are not well defined. (B to F) Dorsal abdominal cuticle (segments A3 to A5) is shown for various *D. melanogaster* genotypes. (B) Canton-5, a wild-type strain of *D. melanogaster*, shows the striped dorsal abdominal pigment pattern typical of this species. (C) Expression of UAS-GFP (green) shows that *pnr-Gal4*, the driver used to ectopically express Ebony and Tan in (D) to (F), activates gene expression in a stripe along the dorsal midline during late pupal development. (D) Ectopic expression of UAS-Ebony caused increased yellow pigmentation. (E) Ectopic expression of UAS-Tan caused increased brown pigmentation. (F) Simultaneous expression of both UAS-Tan and UAS-Ebony resulted in an intermediate phenotype. Cuticle is from 3- to 5-day-old females in all panels except (C), in which cuticle is from a female pupa just before eclosion (stage P15).

involved in pigmentation (13) or a cryptic, small, noncoding RNA encoded by the *tan* intron (18). **Phenotypic consequences of *tan* divergence revealed in transgenic flies.** To determine whether evolutionary changes in the *tan* gene itself are sufficient to affect pigmentation, we inserted transgenes carrying the *D. americana* and *D. novamexicana* *tan* alleles into the *D. melanogaster* genome (19). Both transgenes were integrated at the same site, allowing us to compare pigmentation of flies whose genomes differed only for divergent sites within the transgenes. Both the *D. americana* and *D. novamexicana* alleles of *tan* rescued pigmentation in a *D. melanogaster tan* null mutant (Fig. 4, A to

D), indicating that the transgenes were expressed in *D. melanogaster* and that Tan protein function is (at least largely) conserved. Flies carrying the *D. americana tan* allele had darker pigmentation than flies carrying the *D. novamexicana tan* allele (Fig. 4, C and D; $F = 26.94$, $P < 0.0001$ for abdominal segments A3 and A4, and $F = 6.51$, $P = 0.03$ for the darker A5 segment). This is consistent with the darker pigmentation of *D. americana* relative to *D. novamexicana*. We also compared the phenotypic effects of *D. americana* and *D. novamexicana tan* alleles in

D. americana and *D. novamexicana* themselves by randomly inserting both *tan* transgenes into the genomes of both species. Two independent insertions were recovered for each transgene in each species. In *D. americana*, we were unable to detect a difference in pigmentation between transformed and untransformed flies, presumably because of the already dark pigmentation of this species (see Fig. 1B). In *D. novamexicana*, however, transformant flies carrying the *D. americana tan* transgene (Fig. 4F) were visibly darker than flies carrying the *D. novamexicana tan* transgene (Fig. 4E).

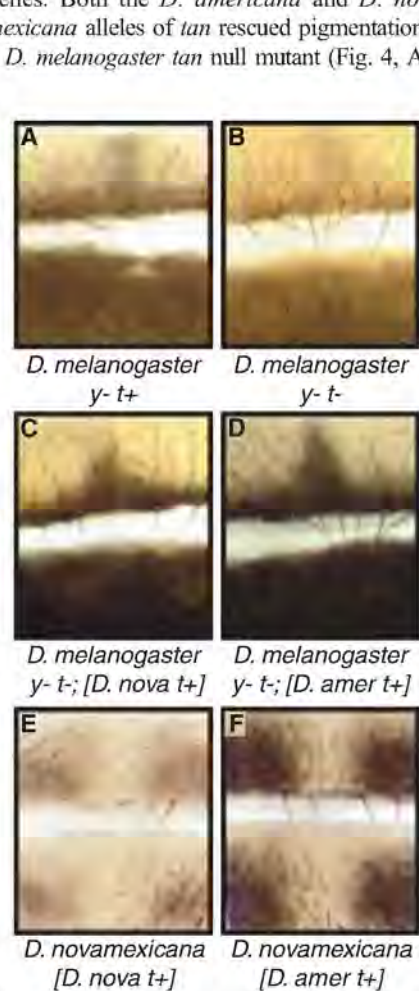


Fig. 4. The *D. americana* allele of *tan* causes darker pigmentation than the *D. novamexicana* allele of *tan*. Transgenes containing *tan* alleles from *D. americana* and *D. novamexicana* were transformed into *D. melanogaster*, *D. novamexicana*, and *D. americana*. In *D. melanogaster*, transgenes were crossed into a genetic background homozygous for null mutations in *yellow* (*y-*) and *tan* (*t-*). The *yellow* mutation was used to lighten pigmentation, making the effects of *tan* transgenes easier to see. (A) *D. melanogaster yellow* (*y-*) mutant, which is wild-type for *tan*. (B) *D. melanogaster yellow, tan* (*y-, t-*) double mutant. (C) *D. melanogaster yellow, tan* mutant carrying the *D. novamexicana tan* transgene (*D. nova t+*). (D) *D. melanogaster yellow, tan* mutant carrying the *D. americana tan* transgene (*D. amer t+*). (E) Wild-type *D. novamexicana* carrying the *D. novamexicana tan* transgene. (F) Wild-type *D. novamexicana* carrying the *D. americana tan* transgene.

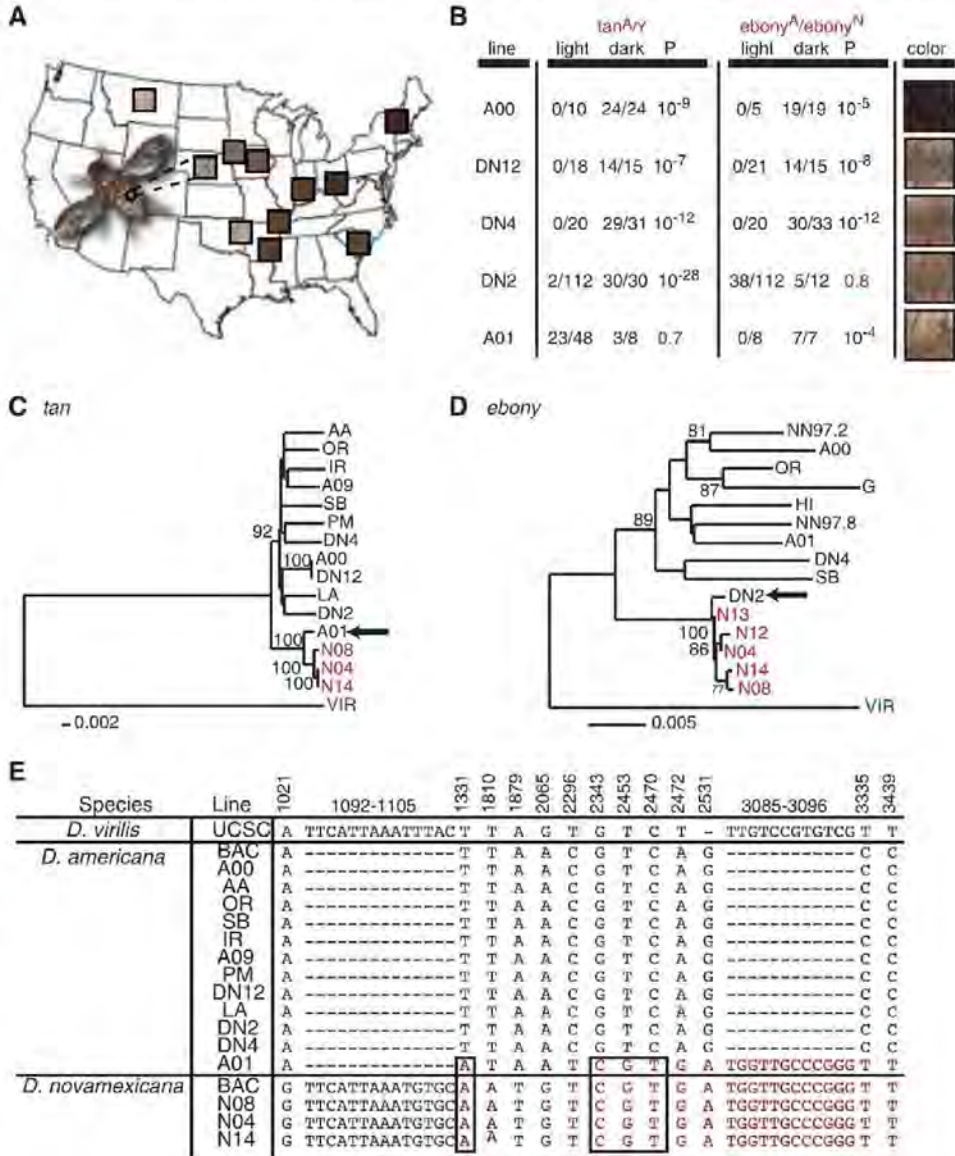


Fig. 5. *ebony* and *tan* QTLs also contribute to polymorphism. (A) Dorsal abdominal cuticle from *D. americana* isofemale lines (table S2) is shown. Eastern populations are darker than western populations. (B) Phenotypes, genotypes, and statistical significance for interspecific QTL mapping experiments. Dorsal abdominal pigmentation of each of isofemale line is shown in the color column; the middle columns show the proportion of male backcross progeny genotyped from the lightest (light) and darkest (dark) pigmentation classes with *D. americana tan* (*tan*^{Am}) and *ebony* (*ebony*^{Am}) alleles. *P* values are from 2×2 Fisher's exact tests of the genotype count data. (C and D) Neighbor-joining trees of *tan* [(C), 7625 base pairs] and *ebony* [(D), 1136 base pairs] from *D. americana* (black), *D. novamexicana* (red), and *D. virilis* (blue) are shown with bootstrap values >75% ($n = 1000$). Branch lengths are to scale. (E) Fixed differences within the 2.7-kb candidate region of *tan*. Sites 889 to 981 and 3521 to 3616 are exons 1 and 2, respectively. The *D. virilis* allele is from the 2005 assembly of the *D. virilis* genome sequence (29). Positions refer to alignment of GQ457336 through GQ457353. Alleles shared between *D. novamexicana* and A01 are red; the derived subset, relative to *D. virilis*, is boxed.

ebony and tan QTLs underlie variable pigmentation within *D. americana*. Pigmentation of *D. americana* is always distinct from that of *D. novamexicana* (3), but the intensity of dark pigmentation varies within *D. americana*. This variation is geographically structured, with *D. americana* captured in the eastern United States visibly darker than those captured from the western part of the species range (3). These pigmentation differences remain visible after rearing flies under common environmental conditions in the laboratory (Fig. 5A), indicating a genetic basis for the pigmentation cline. Sequence variation within *D. americana* at putatively neutral loci shows no population structure (2, 20–24), which suggests that this cline is due to local adaptation.

To determine whether sites linked to *ebony* and/or *tan* contribute to this intraspecific polymorphism, we used *D. novamexicana* alleles as a reference to compare the phenotypic effects of *ebony* and *tan* QTL alleles among lines of *D. americana*. Genetic mapping was performed using four isofemale lines of *D. americana* (A01 collected from Poplar, Montana; DN2, DN4, and DN12 collected from Duncan, Nebraska) with lighter pigmentation than the line of *D. americana* (A00) used previously. *D. americana* females from each strain were crossed to *D. novamexicana* males, F₁ hybrid females were backcrossed to *D. novamexicana* males, and molecular markers in *ebony* and *tan* were genotyped in flies from the lightest and darkest pigmentation classes (fig. S6). Despite their lighter pigmentation, the DN12 and DN4 lines of *D. americana* produced similar mapping results to A00: Both *ebony* and *tan* showed highly significant linkages to loci affecting pigmentation (Fig. 5B). Genotyping 101 males from the DN4 backcross population and fitting their genotypes and phenotypes to a linear model (19) showed that *ebony* (E) and *tan* (T) both had significant additive effects on pigmentation ($F_E = 132.98$, $P_E < 0.0001$; $F_T = 160.85$, $P_T < 0.0001$) with no significant epistatic interaction between them ($F = 3.02$, $P = 0.09$). These additive effects explained 76% of the pigmentation variance in the DN4 backcross population. For DN2, sites linked to *tan* contributed to pigmentation differences between species, but sites linked to *ebony* did not (Fig. 5B). The converse was true for A01: Sites linked to *ebony* contributed to pigmentation differences between species, whereas sites linked to *tan* did not (Fig. 5B).

Taken together, these data reveal three distinct genotypes among *D. americana* lines with a light-pigmentation phenotype. DN2 has alleles linked to *ebony* that appear to be functionally equivalent to those found in *D. novamexicana*; A01 has alleles linked to *tan* that appear to be functionally equivalent to those found in *D. novamexicana*; and DN4 and DN12 have alleles linked to *tan* and *ebony* that appear to be functionally distinct from those found in *D. novamexicana*. It remains to be seen whether the

DN12 and DN4 alleles of these QTLs have the same effect on pigmentation as each other or as the alleles from the darker A00 line of *D. americana*. Two of the three *D. americana* lines (DN2 and DN4) were collected during the same year and DN12 the following year (table S2), which suggests that genetic heterogeneity for pigmentation exists within this local population. This heterogeneity may be caused by gene flow among populations and/or balancing selection within the population.

Shared pigmentation alleles contribute to polymorphism and divergence. The functional similarity observed for the A01 and *D. novamexicana* alleles linked to *tan*, as well as for the DN2 and *D. novamexicana* alleles linked to *ebony*, may result from shared ancestry (i.e., alleles that are identical by descent) or from convergent evolution. Sequences of *tan* and *ebony* from multiple lines of *D. americana* and *D. novamexicana* show that the functional similarity most likely reflects shared ancestry, as the A01 *tan* sequence is more similar to *D. novamexicana* alleles than to other *D. americana* alleles (Fig. 5C) and the DN2 *ebony* sequence is more similar to *D. novamexicana* alleles than to other *D. americana* alleles (Fig. 5D). The DN2 line of *D. americana* also has the same arrangement of the *ebony*-containing inversion ["In(2)b" in (11)] as *D. novamexicana* (fig. S7) (25), further suggesting that pigmentation alleles linked to *ebony* in DN2 and *D. novamexicana* have a common origin.

Sequence variation identifies candidate sites for divergent pigmentation. Sequence similarity between A01 and *D. novamexicana* was found to be highest beginning in the first intron of *tan* and extending 3' of *tan* (fig. S8). Within the 2.7-kb region identified by fine-scale mapping (fig. S3), we observed 13 fixed single-nucleotide differences and two fixed indels between *D. americana* (excluding A01) and *D. novamexicana* (Fig. 5E). The A01 allele of *D. americana* contains the same sequence as *D. novamexicana* at nine of these 13 divergent sites and shares one of the two indels (Fig. 5E, red). Only four of the shared substitutions are derived changes relative to *D. virilis* (Fig. 5E, boxed). Because *D. virilis* has pigmentation similar to *D. americana* (Fig. 1B), we consider these four noncoding changes to be the best candidates for divergent function in this region. Derived changes outside of this region that are also unique to A01 and *D. novamexicana* *tan* may contribute to pigmentation divergence as well. The two amino acid differences between alleles used for fine-scale mapping are polymorphic and are thus unlikely to contribute to fixed differences between species.

A model of pigmentation evolution. Our data reveal the relationship between intraspecific polymorphism and interspecific divergence by showing that the same alleles contribute to pigmentation differences within and between species (fig. S9). These alleles may have been

present in the common ancestor of *D. americana* and *D. novamexicana*, or they may have arisen in *D. novamexicana* and subsequently introgressed into *D. americana* after hybridization. Distinguishing between these two scenarios is notoriously difficult (26–28), yet haplotype sharing between *D. americana* and *D. novamexicana* has been postulated to be due to shared ancestral variation (2). Our data are consistent with this interpretation: Sequences of *D. americana* alleles that appear to have the same function as *D. novamexicana* alleles are basal to these *D. novamexicana* alleles in gene trees (Fig. 5, C and D); there is evidence of recombination (or gene conversion) within the *D. americana* A01 *tan* haplotype (fig. S5A), which argues against a recent introgression event; and *D. novamexicana* is thought to have evolved from a peripheral population of the common ancestor shared with *D. americana* (2). Therefore, we propose that light-pigmentation alleles segregating in this common ancestor became fixed in *D. novamexicana*, contributing to its yellow body color, and continue segregating in *D. americana*, contributing to clinal variation. Additional *D. novamexicana*-like alleles of *D. americana* are needed to further evaluate this model.

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Supporting Online Material

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RNAi in Budding Yeast

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RNA interference (RNAi), a gene-silencing pathway triggered by double-stranded RNA, is conserved in diverse eukaryotic species but has been lost in the model budding yeast *Saccharomyces cerevisiae*. Here, we show that RNAi is present in other budding yeast species, including *Saccharomyces castellii* and *Candida albicans*. These species use noncanonical Dicer proteins to generate small interfering RNAs, which mostly correspond to transposable elements and Y' subtelomeric repeats. In *S. castellii*, RNAi mutants are viable but have excess Y' messenger RNA levels. In *S. cerevisiae*, introducing Dicer and Argonaute of *S. castellii* restores RNAi, and the reconstituted pathway silences endogenous retrotransposons. These results identify a previously unknown class of Dicer proteins, bring the tool of RNAi to the study of budding yeasts, and bring the tools of budding yeast to the study of RNAi.

RNA-silencing pathways contribute to transposon silencing, viral defense, DNA elimination, heterochromatin formation, and posttranscriptional repression of cellular genes (1, 2). In the simplest form of silencing, known as RNA interference (RNAi), the ribonuclease III (RNaseIII) endonuclease Dicer successively cleaves double-stranded RNA (dsRNA) into small interfering RNAs (siRNAs), which are loaded into the effector protein Argonaute to guide the cleavage of target transcripts (1, 3). RNAi arose in an early eukaryotic ancestor and appears to have been conserved throughout most of the fungal kingdom (4, 5) (Fig. 1A). A prominent exception is *Saccharomyces cerevisiae*, a budding yeast that lacks recognizable homologs of Argonaute, Dicer, and RNA-dependent RNA polymerase (RdRP), which in some RNAi pathways produces dsRNA. Indeed, RNAi has been presumed lost in all budding yeasts. Despite this perceived loss, Argonaute genes are present in some budding yeasts (6, 7), including *Saccharomyces castellii*

and *Kluyveromyces polysporus* (both close relatives of *S. cerevisiae*) and *Candida albicans* [the most common yeast pathogen of humans (8)] (Fig. 1A). The presence of these genes in budding yeast has been enigmatic, because other RNAi genes, especially Dicer, have not been found in these species. A similar conundrum appears in prokaryotes, in which certain bacteria have Argonaute homologs yet lack the other genes associated with RNAi or related RNA-silencing pathways (9).

siRNAs in budding yeasts. To search for RNA silencing in budding yeast, we looked for short-guide RNAs, isolating 18- to 30-nucleotide (nt) RNAs from *S. castellii*, *K. polysporus*, and *C. albicans* and preparing sequencing libraries representing the subset of small RNAs with 5'-monophosphates and 3'-hydroxyls (10), which are the chemical features of Dicer products. The small RNAs of *S. castellii* and *K. polysporus* were most enriched in 23-RNAs beginning with U, and those of *C. albicans* were most enriched in 22-nt beginning with A or U (Fig. 1B). These biases were reminiscent of those observed for Argonaute-bound guide RNAs of animals, plants, and other fungi (11–13). Analogous RNAs were not found in *S. cerevisiae*, as expected for a species lacking RNAi (Fig. 1B).

Although some reads from the Argonaute-containing yeasts mapped to ribosomal RNA (rRNA) and transfer RNA (tRNA) and presumably represented degradation intermediates of abundant RNAs, many reads clustered at other types of genomic loci. The loci generating the most reads had sequence homology to repetitive

elements, including long terminal repeat retrotransposons (Ty elements), LINE (long interspersed nuclear element)-like retrotransposons (Zorro elements), and subtelomeric repeats (Y' elements) (Fig. 1C and table S1). Loci of *S. castellii* were also particularly enriched in long inverted repeats; these palindromic loci generated most of the reads with homology to Ty elements (Fig. 1, C and D). In *S. cerevisiae*, essentially all the reads appeared to represent degradation fragments of rRNA, tRNA, and mRNA.

The reads matching inverted repeats suggested origins from paired regions of transcripts that folded back on themselves to form hairpins (Fig. 1D). These inferred hairpins had 100- to 400-bp (base pair) stems, with loops ranging from 19 to >1600 nt. In regions of imperfect duplex, where reads could be mapped unambiguously, the small RNAs tended to match only one genomic strand, which further supported the idea that they originated from hairpin transcripts (Fig. 1D, bottom). Other reads did not map to inverted repeats and, instead, mapped uniquely to both genomic strands in a pattern suggesting that they originated from long bimolecular duplexes involving transcripts from both strands.

Most siRNAs of the fission yeast *Schizosaccharomyces pombe* correspond to the outer repeats of the centromeres and direct heterochromatin formation and maintenance (14). We therefore examined whether any of our sequenced small RNAs matched centromeres. Of the three Argonaute-containing species from which we sequenced (Fig. 1B), only *C. albicans* had annotated centromeres, and almost none (<0.001%) of our *C. albicans* reads matched these genomic loci. Also arguing against a function analogous to that in *S. pombe* is the lack in budding yeasts of recognizable orthologs of the histone H3 lysine 9 (H3K9) methyltransferase Clr4 and recognizable homologs of RdRP, Tas3, Chp1, and the heterochromatin protein HPI-like chromodomain protein Swi6—proteins all necessary for RNAi-dependent heterochromatin in *S. pombe* (14).

When mapped to the genome, the end of one 23-nt RNA was often next to the beginning of another 23-nt RNA, which suggested that endonuclease cleavage simultaneously generated the 3' terminus of one small RNA and the 5' terminus of the next. Consistent with this hypothesis, systematic analysis of the intervals spanning the mapped ends of all 23-nt RNA pairs revealed a clear phasing interval of 23 nt (Fig. 1E). Such phasing implied successive cleavage, beginning

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at preferred starting points. Moreover, pairs from opposite strands had the same phasing interval but in a register 2 nt offset from that of the same-strand pairs. Together, the phasing and offset implied successive cleavage of dsRNA with a 2-nt 3' overhang—the classic biogenesis of endogenous siRNAs by Dicer (3). Therefore, the small RNAs that appeared to derive from regions of dsRNA, i.e., those mapping in clusters to the arms of predicted hairpins and those mapping in clusters to both genomic strands, were classified as siRNAs.

Dicer in budding yeasts. The presence of siRNAs in Argonaute-containing budding yeasts implied that each of these species also had a Dicer-like activity. To assay for this activity, we monitored processing of a long dsRNA added to whole-cell extracts (15). Extracts from *S. castellii*, *K. polysporus*, and *C. albicans*—but not from *S. cerevisiae*—contained an activity that produced 22- to 23-nt RNAs, each preferentially from dsRNA rather than from single-stranded RNA

(Fig. 2A). Moreover, for each extract the small-RNA length matched that of the most abundant length observed in vivo (Figs. 1B and 2A).

Despite the observed Dicer-like activity, a gene with the domain architecture of known Dicers was not found in any budding yeast genome (Fig. 1A) (7). Because we had evidence for cleavage of dsRNA with 2-nt 3' overhangs, a hallmark of RNaseIII activity, we relaxed the search criteria to consider any gene with an RNaseIII domain. *S. cerevisiae* had only one gene, *RNT1*, with a recognizable RNaseIII domain. *RNT1* helps process rRNA and other noncoding RNAs (16), and presumed orthologs were found throughout the fungal kingdom. *S. castellii* had a second RNaseIII domain-containing gene, and a potential ortholog of this gene was found in each of the other Argonaute-containing budding yeasts (Fig. 1A). Anticipating that this second gene encoded the Dicer of budding yeasts, we named it *DCR1*.

To test whether the Dicer candidate is required for siRNA accumulation, we deleted *DCR1* in

S. castellii—the closest relative to *S. cerevisiae* among the sequenced Argonaute-containing species. This procedure required establishing strains and protocols to better enable molecular genetic analysis in this species (15, 17). In the *Δder1* mutant, siRNAs failed to accumulate (Fig. 2B, fig. S2, and table S1). Deletion of the Argonaute homolog, which we named *AGO1*, also reduced siRNA accumulation, as expected if loading into Argonaute protected siRNAs from degradation (Fig. 2B, fig. S2, and table S1). For both mutants, ectopically expressing the deleted gene rescued siRNA accumulation (Fig. 2B). These results indicate that the core components of endogenous RNAi pathways—Dicer, Argonaute, and siRNAs—are present in some species of the budding yeast clade.

In *S. pombe* and other fungi, known Dicer genes resemble those in plants and animals, complete with tandem RNaseIII domains, two or three dsRNA-binding domains (dsRBDs), a PAZ domain, and an N-terminal helicase domain (15, 18, 19)

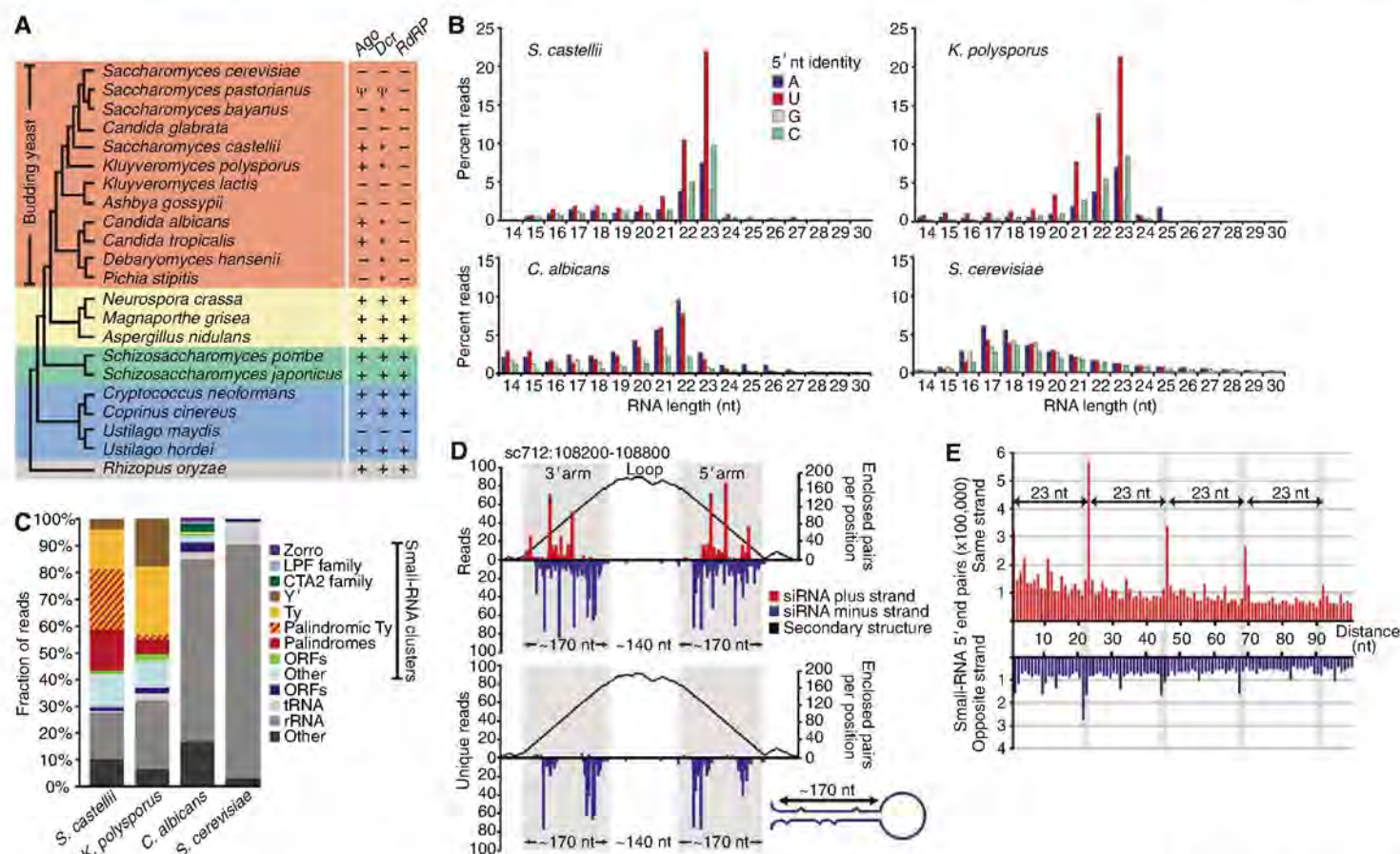


Fig. 1. Endogenous siRNAs in budding yeasts. (A) Cladogram showing Basidiomycota (blue), Zygomycota (gray) and Ascomycota, subdivided into Saccharomycotina (budding yeasts, orange), Pezizomycotina (yellow), and Taphrinomycotina (green) (35, 36). The presence of canonical RNAi genes is indicated (+) [(4, 5) and references therein]. All genomes had an *RNT1* ortholog, and several others had a second RNaseIII domain-containing gene (*), which has Dicer activity in *S. castellii*. Pseudogenes are indicated (†). *S. bayanus*, which had a Dicer but not an Argonaute gene, appeared to lack siRNAs (fig. S1). (B) Length distribution of genome-matching sequencing reads representing small RNAs with the indicated 5' nucleotide. Reads matching rRNA and tRNA are excluded. (C) Classification of loci to which

21- to 23-nt RNAs map, considering those that map to clusters in a pattern suggestive of siRNAs separately from those that do not. (D) A palindromic region generating siRNAs in *S. castellii*. 5' Termini of 22- to 23-nt RNAs were mapped to the genome, and counts (normalized to the number of genomic matches) are plotted for the plus and minus genomic strands. The top considers all reads; the bottom considers those matching the genome at only one locus. The predicted structure of the (–)-strand transcript is represented as a mountain plot (37). (E) Distribution of the genomic intervals separating the 5' termini of sequenced 23-nt RNAs from *S. castellii*. Plotted is the frequency of each interval, when considering all pairs of reads less than 100 nt apart (excluding reads matching rRNA and tRNA).

(Fig. 2C). In budding yeasts, *DCR1* has two dsRBDs, but only a single RNaseIII domain, and no helicase or PAZ domains. Because RNaseIII domains work in pairs to nick both strands of an RNA duplex (19, 20), we suspect that *S. castellii* Dcr1 acts as a homodimer. Dicers of insects, plants, and mammals, which already have two RNaseIII domains, do not homodimerize but do form heterodimeric complexes with cofactors that provide additional dsRBDs (21–23). A homodimeric *S. castellii* Dcr1 complex would already have four dsRBDs, which might obviate the need for such a cofactor.

Except for its second dsRBD, the domain architecture of the budding yeast Dicer resembled that of *RNT1* rather than that of canonical Dicer genes (Fig. 2C). Furthermore, the amino acid sequence of its RNaseIII domain was more similar to that of the *RNT1* RNaseIII domain than to that of any previously identified Dicer RNaseIII domain (Fig. 2D). These observations suggest that budding yeast Dicer might have emerged from a duplication of *RNT1* early in the budding yeast lineage, perhaps coincident with the loss of canonical Dicer. The unusual ancestry and domain structure of *DCR1* might explain why its activity, and thus RNAi more generally, went undetected for so long in budding yeast.

Biochemical analyses of Dcr1 and Ago1. Dicing activity of *S. castellii* extracts was lost in the $\Delta dcr1$ mutant and restored by Dcr1 overexpression (Fig. 2E). To determine whether Dcr1 is active in the absence of *S. castellii* cofactors, we expressed the protein in *S. cerevisiae* and *E. coli* (Fig. 2E). Expression in *E. coli* conferred robust activity, indicating that *S. castellii* Dcr1 is sufficient to dice dsRNA at precise intervals. In other Dicers, the PAZ domain is an essential component of a molecular ruler that imparts cleavage precision (19). The budding yeast Dicers, which lack this domain, must achieve this measuring function differently.

To establish a biochemical link between Ago1 and the siRNAs of *S. castellii*, we sequenced the small RNAs that copurified with tagged Ago1 expressed from its native promoter. Compared with the input RNA, the population of Ago1-associated RNAs was even more enriched for 22- to 23-nt RNAs and was depleted in matches to both rRNA and tRNA, with concomitant enrichment for matches to palindromes, Ty elements, and Y' elements (fig. S3 and table S2). These biochemical results supported the genetic link between *AGO1* and the siRNAs (Fig. 2B) and provided a set of small RNAs suitable for annotating the siRNA-producing loci of *S. castellii* (table S3).

The impact of RNAi on the *S. castellii* transcriptome. To investigate the molecular consequences of RNAi, we performed high-throughput sequencing of polyadenylated RNA [mRNA-Seq (24)] from wild-type, $\Delta ago1$, and $\Delta dcr1$ strains (table S4). The two annotated open reading frames (ORFs) that changed most in RNAi deletion

strains were also the two with the highest density of antisense siRNA reads (Fig. 3A, red points). One was the consensus Y' ORF (fig. S5), which increased more than sevenfold in both deletion mutants. The other was an ORF within a palindromic Ty fragment, which increased more than fourfold in the $\Delta dcr1$ mutant but less in the $\Delta ago1$ mutant. For other ORFs, transcript-abundance changes were modest and not correlated with siRNA density (fig. S6), although changes in $\Delta ago1$ and $\Delta dcr1$ mutants did correlate with each other ($R^2 = 0.39$) (Fig. 3A). This correlation might reflect a general response to the loss of RNAi (although we cannot exclude contributions

of a common response to the hygromycin- and kanamycin-resistance genes used to delete *AGO1* and *DCR1*, respectively).

Because many siRNAs mapped antisense to or outside of ORFs, the mRNA-Seq data revealing the *S. castellii* polyadenylated transcriptome enabled the systematic identification of siRNA precursor transcripts. We focused on three types of siRNA precursors: sense-antisense transcript pairs from ORF loci, partially overlapping mRNAs, and transcripts producing the most siRNA-like reads, regardless of annotation.

The potential for dsRNA composed of sense-antisense transcripts from ORF loci was indicated

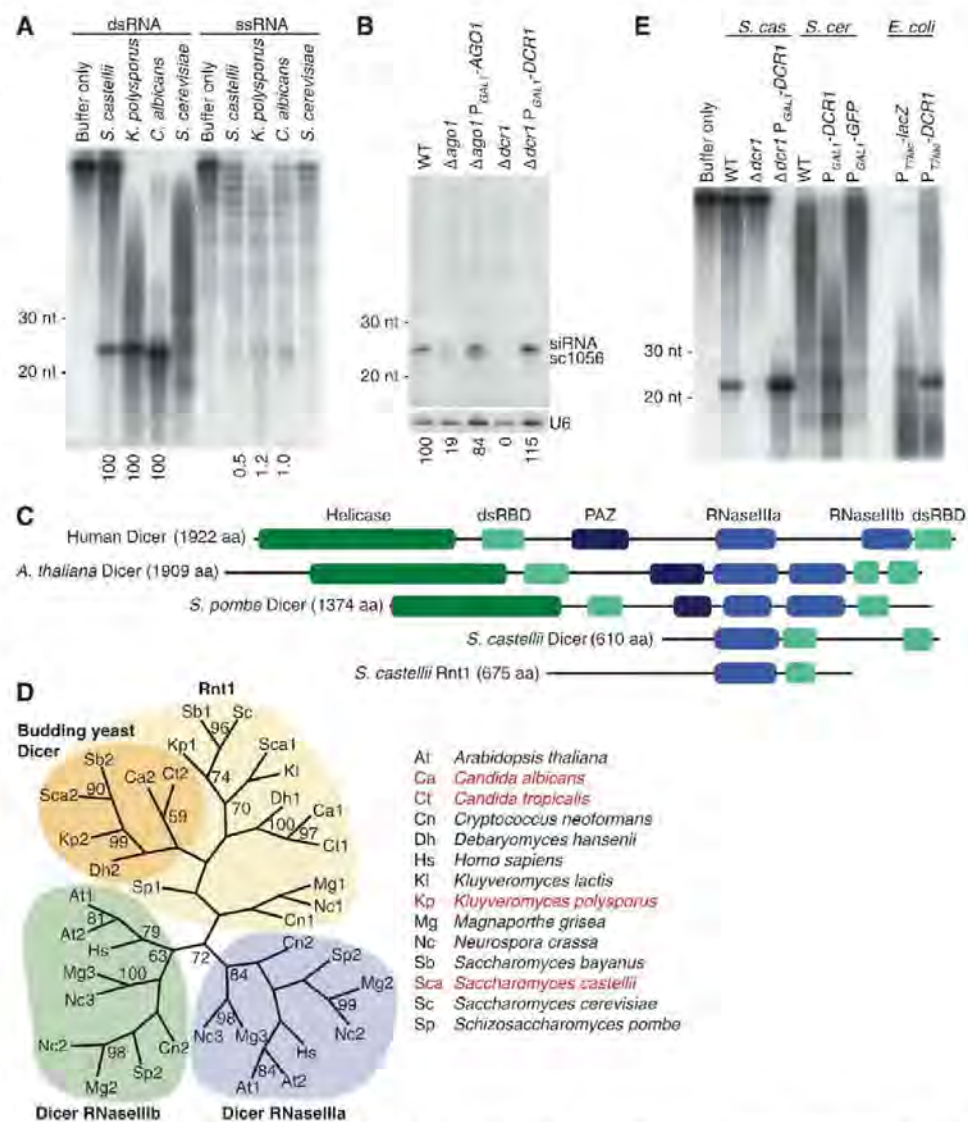


Fig. 2. The Dicer of budding yeast. (A) In vitro processing of radiolabeled dsRNA or single-stranded RNA (ssRNA) in extracts from the indicated budding yeast species. Products were resolved on a denaturing gel. The fraction of product normalized to that observed with dsRNA is indicated below as a percentage. (B) RNA blot probing for an endogenous siRNA (sc1056) in the indicated deletion and rescue strains. The blot was reprobed for U6 small nuclear RNA, and the siRNA percent signal normalized to that of U6 is indicated below. (C) Domain architectures of representative Dicer proteins and the two *S. castellii* proteins containing an RNaseIII domain. (D) Maximum-likelihood tree based on amino acid alignment of RNaseIII domains from Dicer proteins and Rnt1 homologs. Orange shading highlights budding yeast Dicer candidates indicated by asterisks in Fig. 1A. Budding yeast species encoding Argonaute are listed in red. Bootstrap values higher than 50% are shown. (E) In vitro dicing in extracts from recombinant *S. castellii* (*S. cas*), *S. cerevisiae* (*S. cer*), or *E. coli* strains with the indicated deletions and additions, analyzed as in (A).

by widespread low-level antisense transcription of ORFs, with antisense mRNA-Seq tags mapping to over half of all annotated ORFs. Moreover, small RNAs mapped antisense to nearly one-third of ORFs (Fig. 3A) and, as a class, were reduced in RNAi mutants and enriched by Ago1 immunoprecipitation (fig. S3 and table S2). Supporting a precursor-product relation, the abundance of the sense-antisense duplexes (inferred from mRNA-Seq data) correlated with that of

small RNAs deriving from these loci (fig. S7). The most striking example of siRNAs arising from sense-antisense transcript pairs was within the Y' ORF, which was most affected by the loss of the RNAi machinery (Fig. 3, A and B). Y' elements are conserved protein-coding repeats. In *S. cerevisiae*, they are located near both ends of most chromosomes (25) (fig. S7), and synteny suggests analogous locations in *S. castellii* (26). The *S. castellii* elements had a robustly ex-

pressed antisense transcript with many siRNAs mapping to the region of sense-antisense overlap (Fig. 3B).

We considered partially overlapping mRNAs as another potential source of siRNA-generating dsRNA, after using the mRNA-Seq data to extend the 5' and 3' boundaries of 5297 *S. castellii* protein-coding transcripts. Although only 1% of divergent transcript pairs and 7% of tandem transcript pairs overlapped, 78% of convergent transcript pairs overlapped (Fig. 3C) [median overlap of 92 nt at their 3' ends (fig. S7)]. At least 43% of these convergent and overlapping gene pairs (composing 9% of all gene pairs) generated DCR1-dependent siRNAs in the region of overlap (Fig. 3C and fig. S3); one such pair is illustrated (Fig. 3D). A recent study reported pervasive overlapping transcripts in *S. cerevisiae* (27). Our results revealing analogous overlap in *S. castellii* show that, in contrast to previous speculation, this phenomenon is not restricted to RNAi-deficient organisms and is an ancestral feature of these *Saccharomyces* species (15).

We next inferred precursor transcripts without considering whether or not they overlapped ORFs (table S5). A hidden Markov model analyzing the Ago1-associated small RNAs identified the genomic loci producing abundant siRNAs, and analysis of the mRNA-Seq data from *Δdcr1* strains revealed the corresponding transcripts. In addition to recovering the more prolific ORF-overlapping siRNA precursors, this analysis identified the transcript illustrated in Fig. 1D and transcripts of 84 other non-protein-coding siRNA-generating genes of *S. castellii* [annotated as *NCS1*–*NCS85* (tables S3 and S5)]. Transcripts producing fewer siRNAs in RNAi-competent cells changed modestly, but similarly, in both deletion mutants (Fig. 3E), as observed when analyzing only ORF transcripts (Fig. 3A). Transcripts producing the most siRNAs, which were predominantly from palindromic loci, increased dramatically in the *Δdcr1* mutant but were relatively unchanged in the *Δago1* mutant (Fig. 3E and table S5), which indicated that Dcr1 alone was sufficient to reduce these transcripts to wild-type levels. This mode of posttranscriptional down-regulation may be unique to palindromic transcripts, which can fold into hairpin structures that are ideal Dcr1 substrates but refractory to intermolecular pairing with Ago1-associated siRNAs.

Taken together, our results indicate that more than one thousand genomic loci in *S. castellii* generate siRNAs. The consequences of siRNAs derived from the widespread antisense and overlapping transcription in *S. castellii* are unknown. With the exception of the Y' mRNA, the loss of the RNAi machinery did not substantially affect the levels of mRNAs corresponding to these siRNAs (Fig. 3A and fig. S6). Perhaps in other growth conditions the regulatory impact of non-Y' siRNAs might be more pronounced. The specificity for Y'-element regulation could arise from requiring both an abundance of antisense siRNAs and the ability

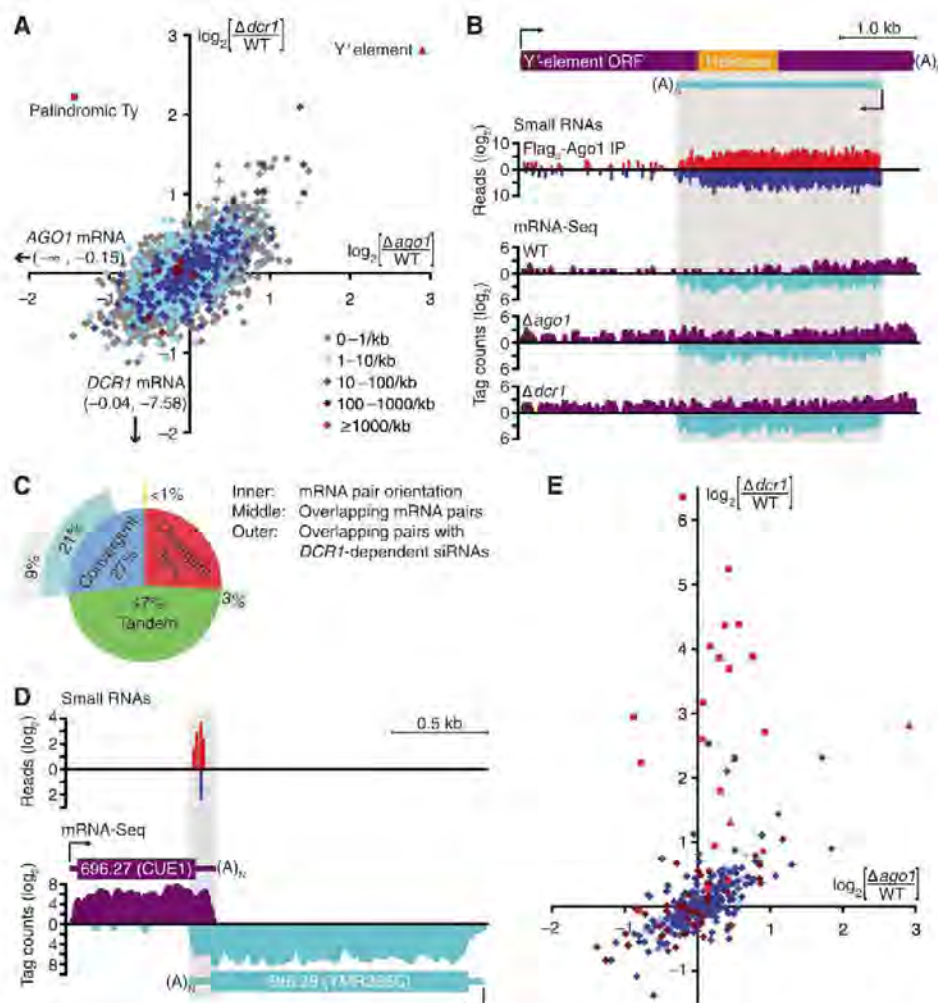


Fig. 3. The impact of RNAi on the *S. castellii* transcriptome. (A) Strand-specific mRNA-Seq analysis of annotated ORF transcripts in wild-type (WT) and RNAi-mutant strains. Plotted is the $\log_2$ ratio of transcript abundance in $\Delta ago1$ versus wild-type (x axis) and $\Delta dcr1$ versus wild-type (y axis). Colors indicate the density (reads per kilobase) of antisense small (22- to 23-nt) RNAs that copurified with Ago1. A Ty ORF fragment (annotated as Scas_712.50) embedded within a palindromic siRNA-producing locus is indicated (square). Annotated Y'-element ORFs were replaced by one consensus Y' ORF (triangle, fig. S5). Because the mRNA-Seq protocol included poly(A) selection, which retains the 3' but not 5' fragments of cleaved mRNAs, we calculated full-length transcript abundance using tags mapping to the 5' half of each ORF. Similar trends were observed when we used tags mapping across the ORF (fig. S4). (B) Analysis of the *S. castellii* Y' element. The numbers of siRNA 5' ends (small RNAs) and mRNA tags (mRNA-Seq) mapping to the consensus Y' element are plotted for each position (sense, above axis; antisense, below axis). (C) Gene-pair organization and overlap in *S. castellii*. (Inner ring) Fraction of neighboring annotated ORFs with the indicated orientation; (middle ring) fraction of transcript pairs with overlapping 3' ends (convergent), overlapping 5' ends (divergent), or continuous transcription in between (tandem); (outer ring) fraction of convergent transcript pairs generating siRNAs in the overlapping region. (D) A pair of convergent transcripts that generate siRNAs in the region of overlap. Plots are as in (B). (E) mRNA-Seq analysis of inferred siRNA-generating transcripts. The plot is as in (A), with the same colors to indicate siRNA-read density and shapes to indicate transcripts mapping to Y' elements (triangle), palindromes (square), and others (diamonds).

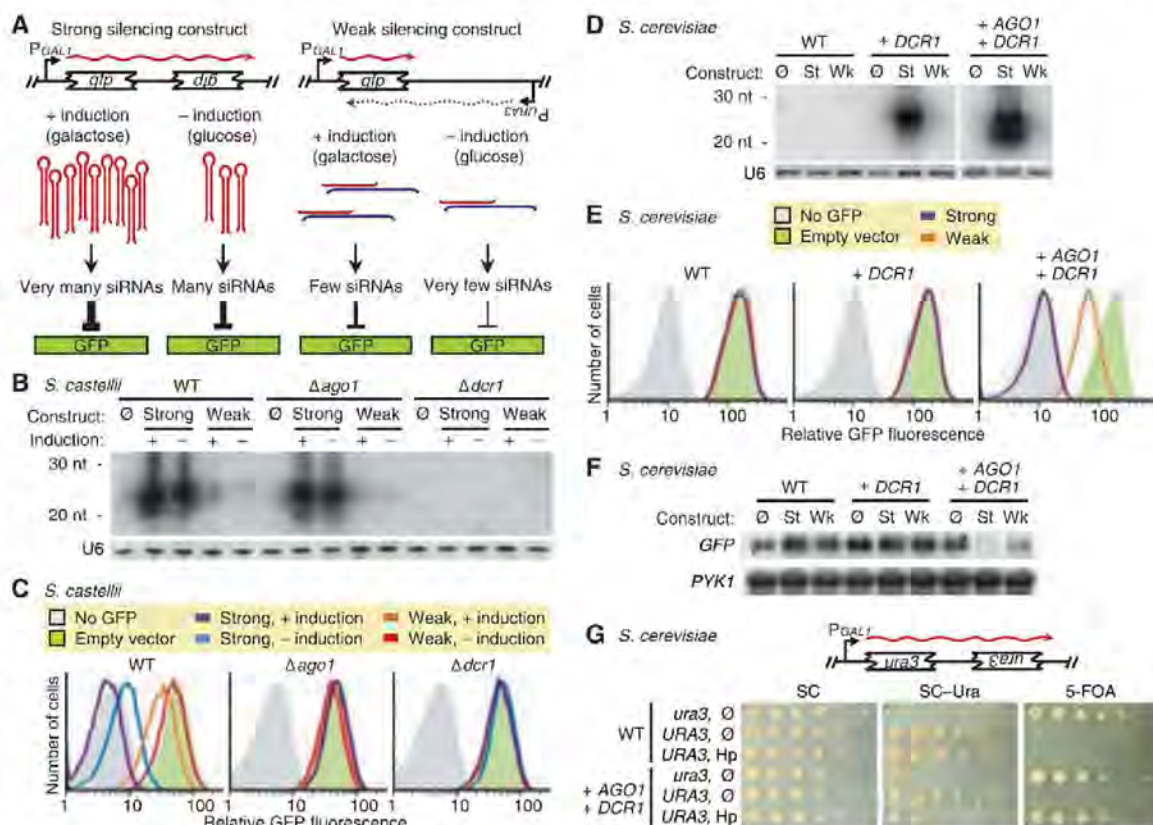
to base pair with a target transcript. Although palindromic loci generate many siRNAs, the hairpin structure of these transcripts might block pairing with siRNAs, and although coding mRNAs are relatively unstructured, most generate only low levels of siRNAs. These two requirements would explain the observed impact of RNAi on the *S. castellii* transcriptome.

Fig. 4. Engineering RNAi in *S. castellii* and *S. cerevisiae*.

(A) Schematic for silencing of a GFP reporter. The strong silencing construct included inverted repeats of a *gfp* fragment and was designed to produce a hairpin transcript (38). The weak silencing construct contained one copy of the fragment, which is transcribed convergently to produce dsRNA. **(B)** RNA blot probing for siRNAs antisense to *GFP*, by using total RNA from the indicated *S. castellii* strains with integrated empty vector (Ø) or silencing construct (strong or weak), either induced with galactose (+) or uninduced (–). The blot was reprobed for U6 small nuclear RNA. **(C)** FACS histograms showing GFP fluorescence in the indicated *S. castellii* strains expressing the indicated silencing constructs. **(D)** RNA blot probing for siRNAs antisense to *GFP* in *S. cerevisiae* strains expressing either no *S. castellii* genes (WT) or the indicated integrated *S. castellii* genes, and either the strong (St), the weak (Wk), or no (Ø) silencing construct. The blot was reprobed for U6 small nuclear RNA. **(E)** FACS histograms showing GFP fluorescence in the indicated *S. cerevisiae* strains expressing the indicated silencing constructs. All strains were induced; silencing from uninduced constructs was similar for the strong construct and undetectable for the weak construct (fig. S9). **(F)** RNA blot probing for *GFP* mRNA in the indicated *S. cerevisiae* strains expressing the indicated silencing

Engineering RNAi in *S. castellii*. To confirm that siRNAs can silence a gene in *S. castellii* and to create tools for monitoring RNAi in budding yeast, we generated two constructs (strong and weak) designed to silence a green fluorescent protein (GFP) reporter gene (Fig. 4A). Both silencing constructs were under the control of an inducible promoter, and each was integrated into

the chromosomes of wild-type, $\Delta ago1$, and $\Delta dcr1$ strains expressing GFP. The two constructs and two induction conditions produced a gradient of GFP siRNAs (Fig. 4B). In cells containing both *AGO1* and *DCR1*, the amount of GFP silencing, as measured by fluorescence-activated cell sorting (FACS), corresponded to the level of GFP siRNAs, with the highest level of siRNA



constructs. The blot was reprobed for *PYK1* mRNA as a loading control. **(G)** Silencing an endogenous gene. *S. cerevisiae* strains containing nonfunctional and functional *URA3* genes (*ura3* and *URA3*, respectively) and expressing the indicated *S. castellii* genes and either the diagrammed hairpin construct (Hp) or no silencing construct (Ø) were tested for Ura3p expression by plating serial dilutions on complete medium (SC), medium lacking uracil (SC–Ura), and medium containing 5-FOA (to which cells producing Ura3p are sensitive).

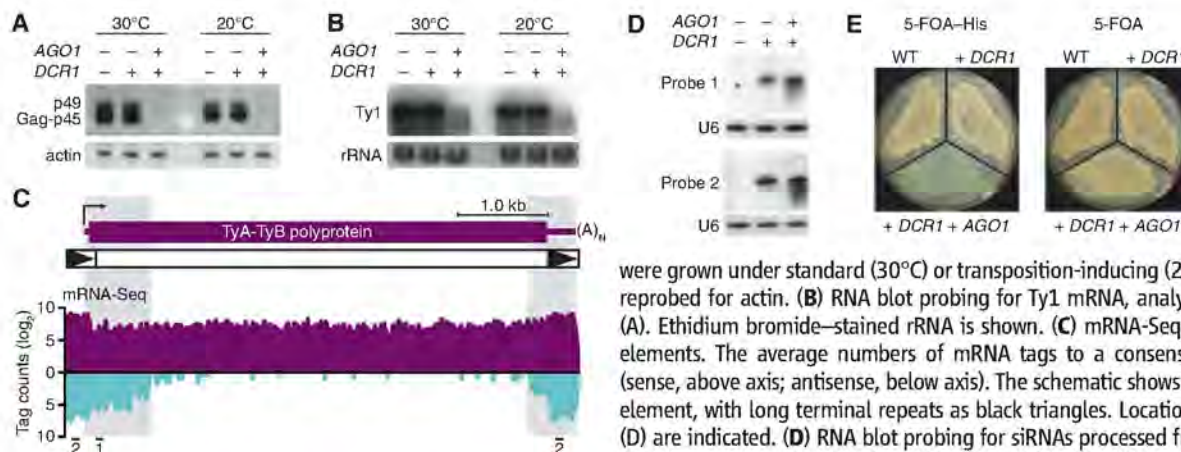


Fig. 5. Silencing of Ty1 retrotransposons by RNAi in *S. cerevisiae*. **(A)** Immunoblot probing for Ty1 Gag protein (p45) and its precursor (p49) (39) in *S. cerevisiae* strains expressing the indicated *S. castellii* genes. Strains

were grown under standard (30°C) or transposition-inducing (20°C) conditions. The blot was reprobed for actin. **(B)** RNA blot probing for Ty1 mRNA, analyzing the same cultures as in (A). Ethidium bromide–stained rRNA is shown. **(C)** mRNA-Seq analysis of *S. cerevisiae* Ty1 elements. The average numbers of mRNA tags to a consensus Ty1 element are plotted (sense, above axis; antisense, below axis). The schematic shows a Ty1 transcript (purple) and element, with long terminal repeats as black triangles. Locations of the two probes used in (D) are indicated. **(D)** RNA blot probing for siRNAs processed from endogenous Ty1 dsRNA. The blot was reprobed for U6 small nuclear RNA. **(E)** *HIS3*-marked Ty1 transposition assay. Cells grow without histidine and are resistant to 5-FOA when the *HIS3*-marked Ty1 element has transposed into the genome and the *URA3*-marked plasmid carrying the original *HIS3*-marked element has been lost (33). Also shown is growth on media selective for plasmid loss but not transposition (5-FOA).

production repressing fluorescence to background autofluorescence (Fig. 4C). As expected, silencing depended on *DCR1* for siRNA production and on *AGO1* for siRNA function (Fig. 4, B and C). These results confirmed that siRNAs could function to silence a gene and demonstrated that the targeted transcript could originate from a locus distinct from that producing the siRNAs.

Reconstitution of RNAi in *S. cerevisiae*. Our observation that some budding yeasts closely related to *S. cerevisiae* contain a functional RNAi pathway suggested that the *S. cerevisiae* lineage lost RNAi recently and that perhaps introducing the two RNAi proteins found in *S. castellii*—Ago1 and Dcr1—could restore the pathway. To test this possibility, we used a GFP-reporter system based on our *S. castellii* system. GFP-positive strains of *S. cerevisiae* were generated that expressed either the strong, the weak, or no silencing construct. Introducing Dcr1 was sufficient to generate some GFP siRNAs from the weak construct and abundant GFP siRNAs from the strong silencing construct (Fig. 4D). When Ago1 and Dcr1 were both present, we observed intermediate silencing with the weak construct and robust silencing with the strong construct (Fig. 4E), with the decrease in fluorescence accompanied by a decrease in mRNA factor of >100 (Fig. 4F and fig. S10). Moreover, a hairpin construct targeting *URA3* reduced growth in the absence of uracil and enabled growth on 5-fluoroorotic acid (5-FOA), which demonstrated that the RNAi pathway reconstituted in *S. cerevisiae* can silence an endogenous gene with phenotypic consequences (Fig. 4G).

The ability to reconstitute RNAi in *S. cerevisiae* by using only Ago1 and Dcr1 raises the possibility that the *S. castellii* RNAi pathway requires only these two proteins. This simplicity would make budding yeast RNAi distinct from all known RNAi pathways, which use additional proteins involved in, for example, Argonaute loading [e.g., R2D2 in *Drosophila melanogaster* (28)] or maturation of the silencing complex [e.g., QIP in *Neurospora crassa* (29)]. The four dsRBDs that would be present in a Dcr1 homodimer might explain the absence of a separate loading factor. Alternatively, overexpression of Ago1, Dcr1, and a hairpin precursor might be sufficient to enact RNAi in *S. cerevisiae*, but they might require additional factors for efficient silencing when expressed at physiological levels in *S. castellii*. Another possibility is that the reconstituted pathway uses components that have been maintained in *S. cerevisiae* since its recent loss of RNAi.

RNAi and transposon silencing. The *Δago1* and *Δdcr1* mutants of *S. castellii* were viable, with no obvious growth disadvantage in minimal or rich media at a range of temperatures; no observed decrease in mating, sporulation, or chromosome stability; and no altered sensitivity to a replication inhibitor (hydroxyurea) or to microtubule-destabilizing agents (thiobendazole

and benomyl). However, both *Δago1* and *Δdcr1* mutants had difficulty retaining introduced plasmids, which showed that the loss of RNAi has detectable phenotypic consequences (fig. S11).

We suspected that budding yeast RNAi might also silence transposable elements. RNAi and related processes silence and eliminate transposons in other eukaryotes (2), and a large fraction of our budding yeast siRNAs corresponded to transposable elements. For example, most *S. castellii* siRNAs mapped to fragments of Ty retrotransposons (Fig. 1C). Despite the abundance of Ty fragments, indicative of former activity in the *S. castellii* lineage (fig. S12), we have not yet found an active retrotransposon in the current, albeit incomplete, *S. castellii* genome sequence. Therefore, to test the effect of RNAi on transposition, we turned to the RNAi-competent *S. cerevisiae* strain.

Compared with the strain with no RNAi genes or the one with only *DCR1*, the RNAi-competent strain had much less Ty1 Gag protein and mRNA (Fig. 5, A and B). The dsRNA triggering this repression of protein and mRNA from native Ty1 elements could have come from elements expressing their own antisense transcripts (30) or from neighboring elements or fragments oriented with potential to produce convergent or hairpin transcripts (31). Analysis of published mRNA-Seq data from *S. cerevisiae* (32) revealed regions with many tags antisense to Ty1 elements (Fig. 5C), and these regions produced siRNAs in *S. cerevisiae* strains containing *DCR1* (Fig. 5D). To examine whether RNAi can suppress retrotransposition, we ectopically expressed a Ty1 element marked with *HIS3*, which enabled transposition to be detected as plasmid-independent complementation of histidine auxotrophy (33). Consistent with our molecular findings for endogenous elements, the RNAi-competent strain permitted much less transposition (Fig. 5E). These results, combined with our sequencing data (Fig. 1C), indicate that a major role of budding yeast RNAi is to silence transposons.

Adding the minimal RNAi components conferred transposon silencing to a species normally lacking the RNAi pathway. The recipient strain had no obvious abnormalities, whereas endogenous transposon protein and mRNA were both drastically reduced, which illustrates the ability of RNAi to preferentially target transposon genes rather than other cellular genes. Although specific for transposable elements, the pathway appears general for any element requiring an RNA transcript—including those it had not previously encountered—by exploiting internally initiated antisense transcripts, as well as the intrinsic propensity of these elements to generate hairpin and convergent transcripts as their genomic load increases.

Concluding remarks. We have uncovered an RNAi pathway present in several different budding yeast species that appears distinct from the well-characterized pathway of fission yeast. The two known components of the pathway have a patchy phylogenetic distribution among budding yeasts (Fig. 1A), indicating that the pathway can

be lost easily. Indeed, if transposon silencing is the critical function of the RNAi pathway, then a species in which transposons have been completely silenced for a long evolutionary period is likely to lose all intact elements and thereby lose selection to retain the RNAi pathway, opening the door to reinvasion. Perhaps also contributing to RNAi loss is its potential inhibition of dsRNA viruses and their associated satellite dsRNAs. In *S. cerevisiae*, the M satellite element of the reovirus-like L-A virus encodes a secreted toxin that kills neighboring cells lacking element-encoded immunity (34). If cells that have lost RNAi are better able to retain this system, they might have a selective advantage despite having lost an efficient transposon-defense pathway.

With the discovery and characterization of the budding yeast pathway, RNAi can be used as a tool to silence genes in *S. cerevisiae*, *S. castellii*, and presumably other budding yeasts. RNAi might be particularly useful in *C. albicans*, an obligate diploid for which both gene deletions and genetic screens are not trivial (8). Even in *S. cerevisiae*, RNAi might have advantages for repressing repetitive gene families. RNAi also enables an inducible repression system that might provide an alternative to existing technologies, which involve either nonphysiological expression of the gene of interest (e.g., the galactose-glucose system) or generation of temperature-sensitive mutations. Perhaps more important, the tools of budding yeast can now be applied to the study of RNAi, either by developing reagents to investigate the endogenous pathway in *S. castellii* or by applying existing technologies to examine the reconstituted pathway in *S. cerevisiae*. As we anticipate a productive future for RNAi research in budding yeasts, we note that if, in the past, *S. castellii* rather than *S. cerevisiae* had been chosen as the model budding yeast, the history of RNAi research would have been dramatically different.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1176945/DC1

Materials and Methods

Figs S1 to S12

Tables S1 to S8

References

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Probing the Magnetic Field of Light at Optical Frequencies

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Light is an electromagnetic wave composed of oscillating electric and magnetic fields, the one never occurring without the other. In light-matter interactions at optical frequencies, the magnetic component of light generally plays a negligible role. When we “see” or detect light, only its electric field is perceived; we are practically blind to its magnetic component. We used concepts from the field of metamaterials to probe the magnetic field of light with an engineered near-field aperture probe. We visualized with subwavelength resolution the magnetic- and electric-field distribution of propagating light.

As light interacts with matter, the force exerted by the electric field on a charge is c/v larger than the force applied by the magnetic field, where v is the velocity of the charge and c is the speed of light. As a result, the response of a material to a magnetic field—its magnetic susceptibility—is a factor 10^{-4} smaller than the ease with which it is polarized, the dielectric susceptibility (1). Only when the charges move extremely fast, such as in relativistic plasmas (2, 3), can the magnetic and electric coupling become comparable. In atomic systems, even though the magnetic dipole coupling is extremely weak, it is important for fundamental tests of the standard model of particle physics (4). Magnetic light-matter interaction has been accomplished using artificial “magnetic” atoms. By tailoring

the geometry of such subwavelength metallo-dielectric structures (so-called metamaterials), effective magnetic coupling is achievable in the microwave regime (5, 6). Photonic nanostructures that resonantly respond to the magnetic field at optical frequencies can now be fabricated (7–11). This magnetic resonance can be exploited to study fascinating phenomena, such as negative index of refraction (10), super-lensing (12), and cloaking (13, 14). Whereas many advances have been made in the control of light-matter coupling by magnetic means, the possibility of directly probing the magnetic field at optical frequencies has not yet been explored. The ability to directly probe the magnetic field of light would of course be beneficial to studies on metamaterials.

We used a near-field aperture probe, designed following split-ring resonator concepts, to detect the magnetic field at optical frequencies. Our probe was used to map the amplitude and phase of the magnetic field of propagating light. By simultaneously measuring the electric field as well, both constituent components of light (that is, the magnetic and the electric) can be mapped with sub-wavelength resolution.

We fabricated a nanostructured metallo-dielectric probe to detect the magnetic field at optical frequencies. A subwavelength aperture was created at the end of a tapered aluminum-coated single-mode fiber by means of focused ion-beam milling (15). An air gap of 40 nm was opened with focused ion-beam milling in the coating. Such a split-probe is shown in the lower image of Fig. 1B. We compare the optical signals measured with a split-probe with those measured with a standard, cylindrically symmetric, coated probe (Fig. 1B, top) that was used in the past (16, 17). To ensure that we probed the magnetic field rather than some signal caused by other electric-field components, a well-characterized single-mode Si_3N_4 ridge waveguide was used as a test structure (18, 19). Linearly polarized light from a diode laser, tuned to a wavelength of 1550 nm, was coupled to the transverse electric (TE) mode of the waveguide. A fraction of the light in the waveguide is reflected by the end-facet, which sets up a standing wave inside the waveguide. Any standing wave will exhibit a spatial amplitude modulation in both the electric- and magnetic-field components of the light. The modulation of the amplitudes of the electric and magnetic components are always shifted in space with respect to each other by half a period (20). This modulation is therefore ideal to demonstrate that the magnetic field is being probed.

A phase- and polarization-sensitive near-field microscope was used for scanning and collecting the light (Fig. 1A) (21). The aperture of the probe couples the evanescent field of the light propagating through the waveguide to the probe fiber (22). The light coupled into the probe fiber is mixed with light in the reference branch of a Mach-Zehnder interferometer. Subsequently, the two orthogonal polarizations in the fiber were separated by a polarizing beamsplitter and simultaneously detected with a heterodyne scheme. By raster-scanning the probe 20 nm above the sample, the amplitude and the phase distribution of

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the probed fields were obtained. The high symmetry of the standard probe can be used to distinguish the two in-plane components of the electric field of propagating light (17). As a result of the symmetry of the aperture, the in-plane components of the electric field couple to the orthogonal modes of the probe fiber and are detected in the two separate channels of the microscope (Ch1 and Ch2). For a formal description of what the probe measures, we refer to the supporting online material (SOM) (18). To probe the magnetic field of light, the magnetic field needs to be converted to an electric field before it can be measured at the detector. To achieve this conversion, the symmetry of the conventional probe needs to be broken. In the split-ring probe used in this work, this symmetry was broken by introducing a single gap in the side of the metal coating. This is crucial because a full ring or a ring with two opposing gaps would not exhibit the optical bianisotropy required for converting the magnetic to an electric field (23, 24). Because the width of the air gap is much smaller than the wavelength of light inside the waveguide, the sensitivity to gradients in the electric field (7) is strongly suppressed.

The waveguide was first characterized by the performance of near-field measurements with a standard probe (Fig. 2A). Light propagates along $\hat{x}$, the electric field is along $\hat{y}$, and the magnetic field is along $\hat{z}$. Because the waveguide contains only a weakly guided TE mode, the longitudinal component of the electric field is negligible (18, 25). The probe can be considered as a subwavelength metallic ring parallel to the sample surface because of its cylindrical symmetry and the extension of

the evanescent fields in air of only ≈ 100 nm (25). The evanescent electric-field component E_y induces a dipolar charge distribution in the probe (SOM text). This induced oscillating dipole moment p_y couples to a propagating mode in the probe fiber and can be detected at the other end of the fiber. The magnetic field B_z instead generates a circular current in the ring, as described by Faraday's law. As a result, the probe exhibits a magnetic dipole moment m_z , in analogy with the magnetic response of a metallic cylinder (5). However, the radiation pattern of the magnetic dipole lies in the xy plane, and the cylindrical symmetry of the system forbids coupling of this magnetic dipole to the propagating modes in the probe fiber that propagate along $\hat{z}$. Therefore, the fields detected with a standard probe are the in-plane electric fields (17, 26).

Figure 2B shows the line traces of the amplitude detected on Ch1 (red) and Ch2 (green), which were obtained by scanning the probe along the center of the waveguide. Because the only nonvanishing component of the electric field is along $\hat{y}$, we can attribute the signals detected by Ch1 and Ch2 to E_y and E_x , respectively (Fig. 2A). Because in the center of the waveguide the longitudinal component (E_z) vanishes (25), Fig. 2B allows us to infer the experimental extinction ratio of the two polarization channels. Because the ratio between the two amplitude signals is $\sim 1/20$ (in terms of intensity $\sim 1/400$), we were able to separate the two polarization states. As expected, a clear spatial amplitude modulation is evident in both channels. Because the spatial period (~ 500 nm) is half of a wavelength in the waveguide (18), this modulation is attributed to the standing wave

caused by the interference between the forward-propagating light and the small fraction of light that is reflected by the end-facet of the waveguide. The maxima of the amplitude modulation in Ch1 and Ch2 are not shifted in space because both channels probe electric fields.

The sensitivity of the probe to the various field components of light changes drastically when the split probe is used. The air gap is oriented along $\hat{y}$ (Fig. 2C). In analogy to the standard probe, E_y and B_z will induce in the split probe an electric and magnetic dipole moment p_y and m_z , respectively. Similar to the cylindrical probe, the dipole moment p_y will generate an optical signal in Ch1. However, because of the air gap the magnetically induced current cannot flow completely around the ring and will produce a time-varying dipolar charge distribution across the gap. This in-plane magnetically induced electric dipole moment can now couple to the fiber of the probe. Because the polarization of this radiation is along $\hat{x}$, the signal corresponding to B_z will be detected by Ch2. Hence, the optical signals with an electric and magnetic origin are detected in Ch1 and Ch2, respectively.

Figure 2D shows the measured amplitude of the Ch1 and Ch2 signals obtained with a split probe. In contrast to Fig. 2B, the signals in the two channels now have comparable magnitude. The standing wave induced amplitude modulation of the two signals is roughly equal. However, the most important difference between Fig. 2B and 2D is that the maxima of the two standing waves are shifted in space by half a period. It is well known that in a standing light wave, the amplitude of the magnetic field is shifted in space by half of a period with respect to the amplitude of the electric field (20). The spatial shift of the local maxima in Fig. 2D is therefore a clear signature that Ch2 detects the light generated by the coupling with B_z . Thus, we have probed the out-of-plane component of the magnetic field.

To verify our claim of magnetic sensitivity of the probe, we performed an additional check. The same split-probe was used to measure on a waveguide oriented along $\hat{y}$ while keeping the air gap oriented along $\hat{y}$ (Fig. 2E). In this configuration, the electric field is along $\hat{x}$ rather than $\hat{y}$, and thus it should be detected by Ch2. However, B_z should also be probed by Ch2 because the orientations of the probe and, consequently, of the magnetically induced electric dipole moment have not changed. This means that the channel with the higher signal should now be Ch2. This was indeed experimentally observed, as shown in Fig. 2F. Although the ratio between the Ch1 and Ch2 signals is only ≈ 0.27 (in terms of intensity, ≈ 0.07), it was higher than expected (see Fig. 2B). We attribute this to a minute in-plane rotation of the air gap with respect to $\hat{y}$. When the air-gap is not perfectly aligned with $\hat{y}$, the probe projects a fraction of B_z on Ch1. More important, because in this configuration the split probe does not separate E_x and B_z , the amplitude maxima of the two channels are no

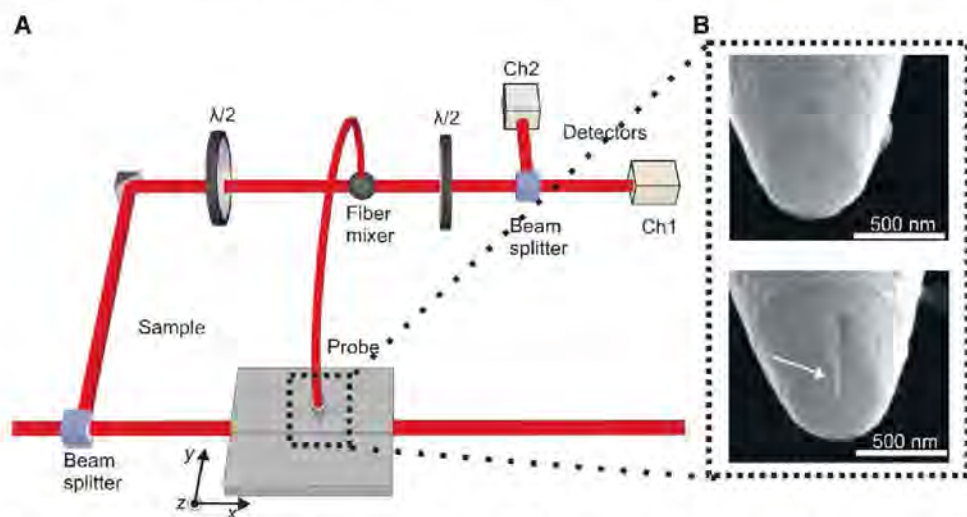


Fig. 1. (A) Schematic of the phase-sensitive near-field microscope. The near-field probe, indicated by the dashed box, is scanned 20 nm above the sample and collects the evanescent field of the light inside the waveguide. The light is mixed with light from a reference branch. The resulting light is split by a polarizing beamsplitter, and the two orthogonal polarizing components are detected with a heterodyne scheme. By suitably choosing the orientation of the two $\lambda/2$ waveplates, we can relate the signal at the two detectors, called Ch1 and Ch2, with the fields present in the sample. **(B)** A scanning electron micrograph of two aluminum-coated near-field probes. For both probes, the coating thickness is 150 nm, and the aperture diameter is 200 to 230 nm. (Top) The highly cylindrical standard probe. (Bottom) A split probe in which an air gap in the metal coating (arrow) has been created.

Fig. 2. (A), (C), and (E) Schematic of the performed experiments. In the upper part, top views are shown. The “ridge” has been colored differently for clarity. In the lower part, cross-sections in the plane perpendicular to the substrate are shown. The red and green lines correspond to Ch1 and Ch2, respectively. (A) The standard probe is depicted in gray as a metallic ring. (C) The split probe is shown as a metallic split ring. (E) Same configuration as (C), but the waveguide is rotated by 90°. (B), (D), and (F) Line traces of the amplitude obtained by scanning a standard probe in the configuration shown in (A), a split probe in the configuration shown in (C), and a split probe in the configuration shown in (E), respectively, along the waveguide. (B) Both of the line traces, which are normalized to the maximum of Ch1, show a standing-wave component. (D) The line traces are normalized to the maximum of Ch2. The Ch2-detected signal is comparable with Ch1. We associate the Ch2-detected signal with B_z . (F) The line traces are normalized to the maximum of Ch2. Both E_x and B_z are projected along $\hat{x}$ and thus are detected by Ch2.

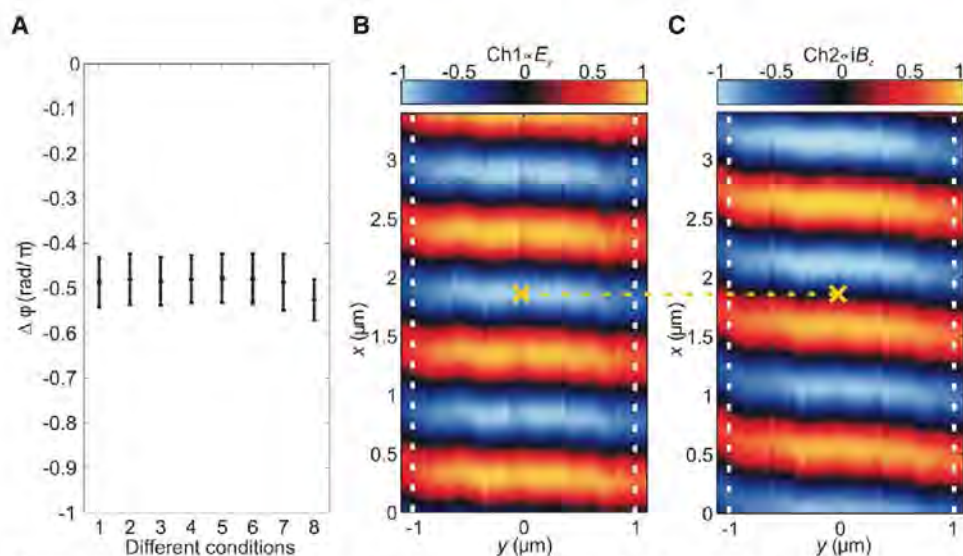
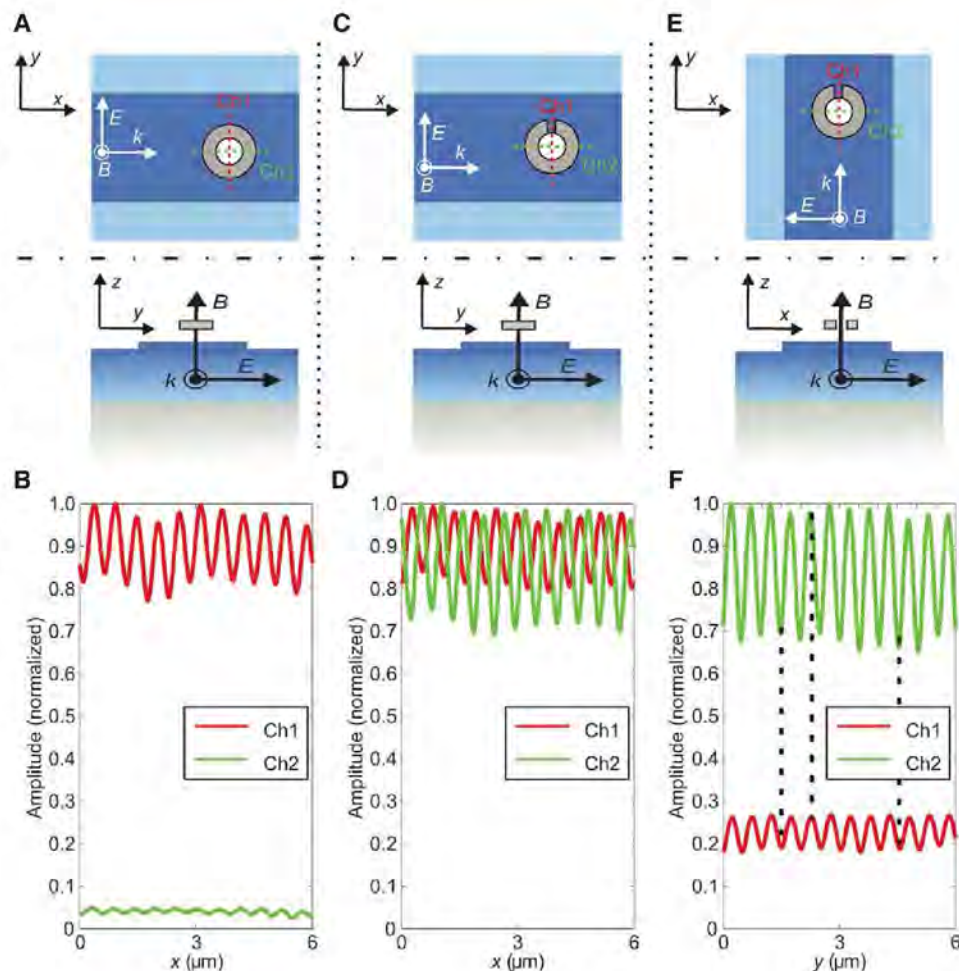


Fig. 3. (A) Phase difference $\Delta\phi$ between the complex signals of Ch1 and Ch2 for the measurement of Fig. 3B for different experimental conditions. (B) and (C) Normalized distributions of $\text{Ch1} \propto \text{Re}(E_y)$ and $\text{Ch2} \propto \text{Re}(iB_z)$, respectively. The images have been obtained by raster-scanning the split probe in the configuration shown in Fig. 2C over an area 2.2 by 3.4 μm^2 . The white dashed lines represent the position of the waveguide. The green dashed line is a guide to the eye that indicates the $\pi/2$ phase shift of the two wave fronts.

longer shifted in space with respect to each other, as indicated by the dashed lines in Fig. 2F.

In the above, we only considered the amplitude of the detected signals. To understand the

working of the split-ring probe, we also have to analyze the phase difference $\Delta\phi$ between the signals in Ch1 and Ch2. As mentioned above, the magnetic-field component of light induces a

current in the ring, which in turn induces a time-varying charge distribution across the gap. Considering that we are far below the resonance frequency of the split probe [on the basis of methods for split-ring resonators (5), we estimate a resonant wavelength of $\lambda_o = 1300$ nm and a width $\Delta\lambda_o = 50$ nm for the resonance], the current in the ring should be in phase with the driving magnetic field. The resulting electric dipole moment along $\hat{x}$ will oscillate 90° out of phase with the current and thus 90° out of phase with the driving magnetic field (SOM text). In short, the probe will respond to B_z with an electric dipole moment $p_x \propto iB_z$, which is analogous to the magnetic response of split-ring resonators (27, 11). To find the expected phase difference between the signals corresponding to the electric and magnetic field (E and B , respectively), we use the curl equation $\nabla \times \mathbf{E} = -\partial\mathbf{B}/\partial t$. We know that for a plane wave traveling in the positive x direction, $\omega B_z = -kE_y$, where ω is the optical frequency and k is the wave number of the light. Thus, B_z has a π phase difference with respect to E_y . Given the additional $\pi/2$ phase shift induced by the probe, the signal detected in Ch2 should be $\pi/2$ out of phase with the E_y signal detected in Ch1. Figure 3A shows for different probes and setup conditions (such as the strain on the fiber and the orientation of the connectors) that we indeed consistently measure a constant phase difference $\Delta\phi = -\pi/2$

(with a spread of 10%) between Ch1 and Ch2, demonstrating that whereas Ch1 probes E_z , Ch2 corresponds to iB_z .

In Fig. 3, B and C, the distributions of the raw data measured in Ch1 and Ch2 are shown, collected by raster-scanning the split probe in the experimental configuration of Fig. 2C. As argued above, Ch1 and Ch2 correspond to the real part of E_z and iB_z , respectively. The white dashed lines represent the position of the waveguide. A closer look at the wavefronts in Fig. 3, B and C, shows again that because of the detection mechanism of the probe, the two signals exhibit a phase shift of $-\pi/2$, where the yellow dashed lines can be used as a guide to the eye (18). These images demonstrate that we simultaneously visualize with phase-sensitivity and subwavelength resolution the magnetic- and electric-field distribution of light propagating through a ridge waveguide. The magnetic field in Fig. 3C has been directly acquired rather than retrieved by inserting the electric-field distribution in the above-mentioned curl equation (28, 29). The simultaneous spatially resolved probing of both magnetic and electric fields should open up new fascinating research directions. By redesigning the geometry of the probe, it should be possible to detect other components of the light field besides the three shown in this work. A suitable combination of probes should allow one to fully unravel the electromagnetic field in photonic nanostructures. Furthermore, the split-probe can be used as a movable split-ring resonator, and thus we can explore the local coupling between nano-objects that resonantly respond to the mag-

netic field at optical frequencies, such as splitting resonators, double fishnet structures, and stereometamaterials.

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Characterization of a Rhodium(I) σ -Methane Complex in Solution

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Numerous transition metal-mediated reactions, including hydrogenations, hydrosilations, and alkane functionalizations, result in the cleavage of strong σ bonds. Key intermediates in these reactions often involve coordination of the σ bond of dihydrogen, silanes (Si-H), or alkanes (C-H) to the metal center without full scission of the bond. These σ complexes have been characterized to varying degrees in solid state and solution. However, a σ complex of the simplest hydrocarbon, methane, has eluded full solution characterization. Here, we report nuclear magnetic resonance spectra of a rhodium(I) σ -methane complex obtained by protonation of a rhodium-methyl precursor in CDCl_2F solvent at -110°C . The σ -methane complex is shown to be more stable than the corresponding rhodium(III) methyl hydride complex. Even at -110°C , methane rapidly tumbles in the coordination sphere of rhodium, exchanging free and bound hydrogens. Kinetic studies reveal a half-life of about 83 minutes at -87°C for dissociation of methane (free energy of activation is 14.5 kilocalories per mole).

In the spectrum of interactions between transition metals and ligands, the so-called σ complexes, in which an electron pair already involved in the sigma interaction between two light atoms serves as a ligand for a transition metal complex, have taken the longest time to be exposed and elaborated (Fig. 1). In the molecular hydrogen complexes that constitute the simplest class of such σ complexes, the H-H

bonding electrons of an intact H_2 molecule act as a ligand to a transition metal center, creating a three-center, two-electron bond (1). First recognized in 1984, numerous examples of these η^2 -dihydrogen, (or σ -hydrogen) complexes have now been isolated and structurally characterized, and such species are now proposed to be key intermediates in many hydrogenation and hydro-

Relative to η^2 - H_2 complexes, the synthesis and characterization of coordination compounds with saturated hydrocarbons acting as ligands, so-called σ -alkane complexes, have been particularly challenging (2–4). Not only is the strong nonpolar C-H σ bond a weak donor, but steric repulsions between the alkyl group and the metal center impede the close approach of the ligand to the metal center (5). Closely related to this interaction between an alkane and a metal are the now well-established intramolecular three-center, two-electron $\text{M}\cdots\text{H}\cdots\text{C}$ bonds of alkyl agostic complexes (6, 7). In these models for σ -alkane complexes, a C-H bond of an ancillary ligand is ideally positioned to interact with a metal center in a chelate-type interaction as shown in Fig. 1.

The existence of transition metal σ -alkane complexes as transient intermediates has been inferred by isotope scrambling studies and inverse kinetic isotope effects in the reductive

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elimination reaction of alkyl hydride complexes (8, 9). The generation and spectroscopic detection of complexes with an untethered hydrocarbon acting as a ligand to a metal center have typically required specialized techniques (Fig. 2A) (2, 4). In the classic work of Perutz and Turner, a methane complex was inferred by changes in the ultraviolet-visible spectrum in experiments where $\text{Cr}(\text{CO})_5$ was photogenerated from $\text{Cr}(\text{CO})_6$ in a methane matrix at 12 K (10). Evidence of alkane coordination in fluid solution was initially provided by laser flash experiments that liberate a ligand from the metal center to generate a coordinately unsaturated complex, which is then

trapped by alkane solvent (11). These transient species rapidly revert back to starting material (Fig. 2A). A similar strategy has been used to generate and characterize σ complexes that undergo a subsequent unimolecular oxidative addition reaction (12).

Time-resolved infrared spectroscopy (TRIR) study of $\text{Cr}(\text{CO})_5(\text{cyclohexane})$ provided the initial more-detailed spectroscopic characterization of an alkane complex (13). Recently, George and co-workers used TRIR to detect metal carbonyl infrared absorbances of transiently stable metal carbonyl σ -methane and σ -ethane complexes generated in the supercritical alkane at room temper-

ature (14). Nuclear magnetic resonance (NMR) data were obtained for σ -alkane complexes of rhodium in remarkable studies by Ball and co-workers. The alkane complexes, $\text{Cp}^*\text{ReL}(\text{CO})(\text{alkane})$ [Cp^* is $\eta^5\text{-C}_5\text{H}_5$, $\eta^5\text{-C}_5\text{Me}_5$ (Me indicates methyl), or $\eta^5\text{-C}_5\text{H}_4\text{Pr}$; L is CO or PF_3 ; alkane is $n\text{-C}_5\text{H}_{12}$, $n\text{-C}_7\text{H}_{16}$, $c\text{-C}_5\text{H}_{10}$, $c\text{-C}_6\text{H}_{12}$, or $i\text{-C}_4\text{H}_{10}$], were generated by low-temperature photodissociation experiments in pure alkane solvents (15). Although these thermally unstable species were produced in modest yields, the σ -alkane complexes were well characterized by low temperature ^1H and ^{13}C NMR spectroscopy (16). The very low boiling points of methane and other lighter alkane solvents precluded their study by this method. None of these species was amenable to analysis by single crystal x-ray diffraction (17–19).

We report here the full NMR characterization of a transition metal σ -methane complex in solution. In this case, the methane ligand is generated directly in the coordination sphere of the transition metal by protonation of the methyl precursor, a strategy illustrated in Fig. 2B. The σ -methane complex is at lower energy than the methyl hydride complex for this system, permitting spectroscopic characterization of the coordinated methane ligand.

We recently reported the isolation of a 16-valence electron iridium(III) hydrido methyl cation, $[(\text{PONOP})\text{Ir}(\text{H})\text{CH}_3][\text{B}(\text{Ar}^F)_4]^+ [\mathbf{1}(\text{-H})\text{CH}_3]^+$ {where PONOP is 2,6-($t\text{Bu}_2\text{PO}$) $_2\text{C}_5\text{H}_3\text{N}$; $t\text{Bu}$ is $\text{C}(\text{CH}_3)_3$; and $\text{B}(\text{Ar}^F)_4$ is $\text{B}[3,5\text{-(CF}_3)_2\text{C}_6\text{H}_3]_4$ }, that exhibits unexpected stability toward methane elimination [$t_{1/2} = 3$ hours at 23°C ; free energy of activation ($\Delta G^\ddagger$) = 22 kcal/mol] (20). Rapid interchange between the Ir-H and Ir-CH₃ protons of $\mathbf{1}(\text{-H})\text{CH}_3^+$ [observed rate constant (k_{obs}) = $4.2 \pm 0.8 \text{ s}^{-1}$ at -105°C ; $\Delta G^\ddagger = 9.3 \pm 0.4 \text{ kcal/mol}$], observed by NMR spectroscopy, established the reversible formation of a transient iridium(I) σ -methane complex (20). Density functional theory (DFT) computational studies indicate that $[(\text{PONOP})\text{Ir}(\text{CH}_4)]^+ [\mathbf{1}\text{-CH}_4]^+$ lies only ~5 kcal/mol higher in energy than the $\mathbf{1}(\text{-H})\text{CH}_3^+$ ground state (21). The small ground state free energy

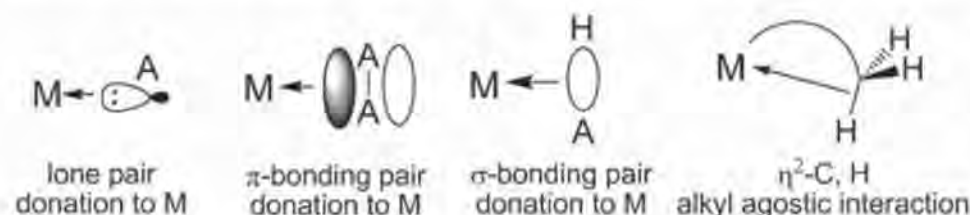


Fig. 1. Transition metal (M)-ligand bonding and alkyl agostic interactions; the back-bonding component is not shown.

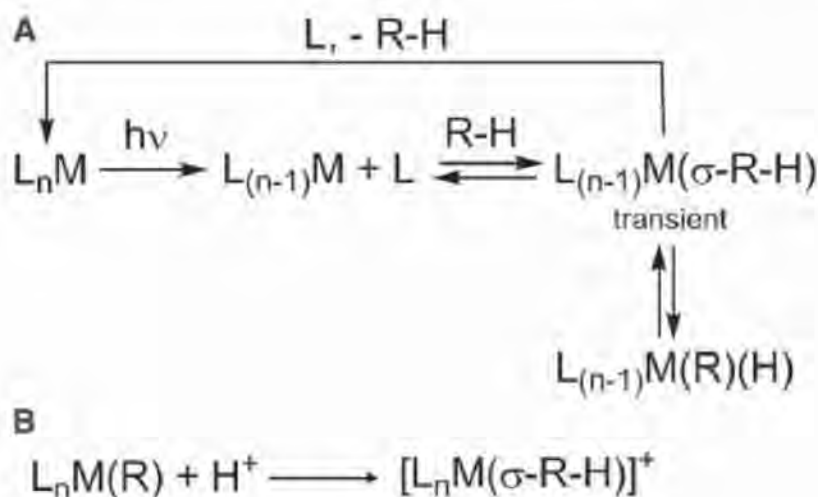


Fig. 2. Strategies for preparing alkane complexes.

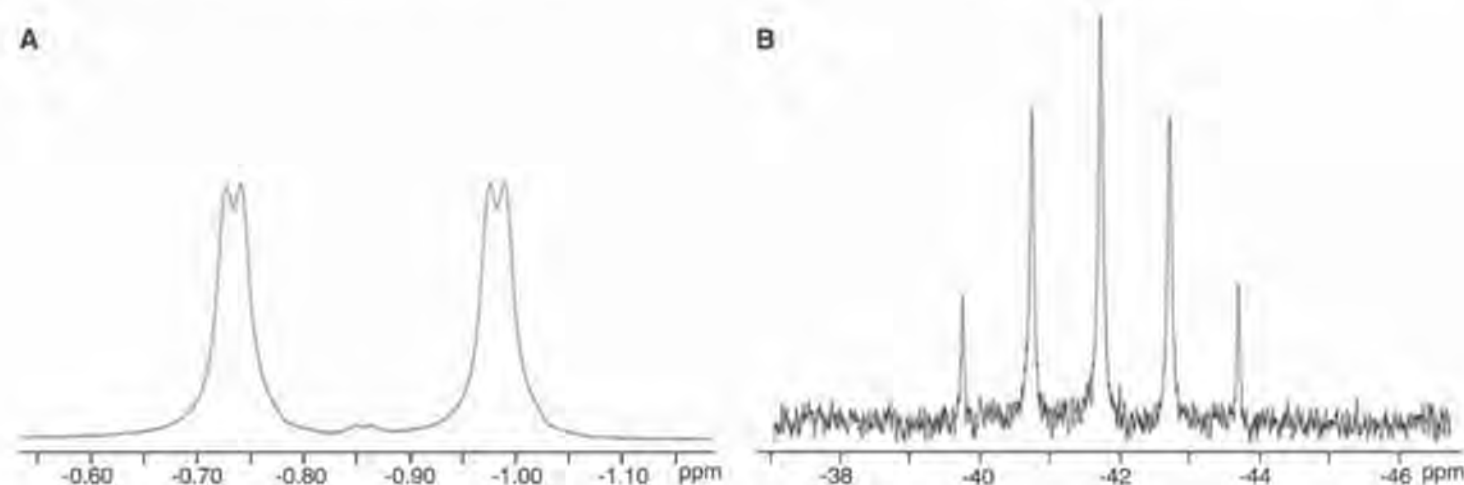


Fig. 3. (A) Partial ^1H NMR and (B) ^1H coupled ^{13}C NMR spectra of $2\text{-}^{13}\text{CH}_4^+$ in CDCl_2F at -110°C .

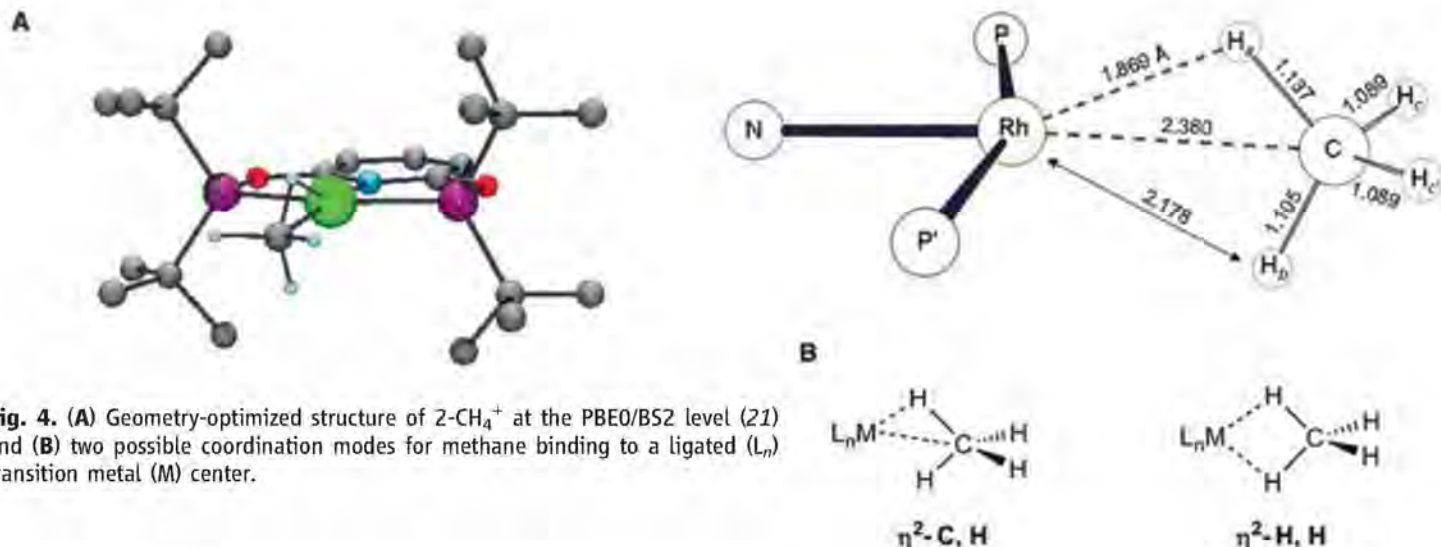
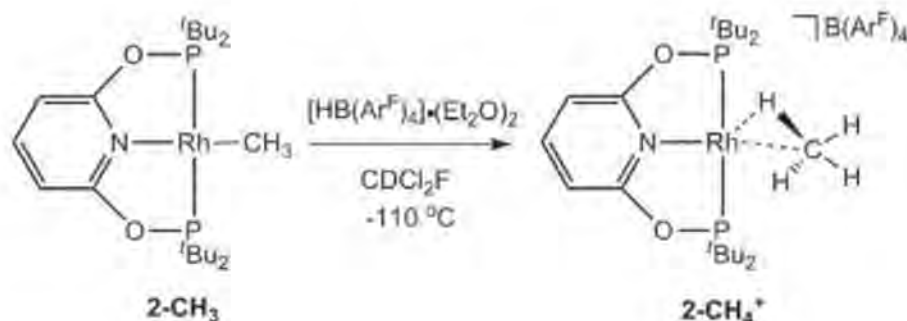


Fig. 4. (A) Geometry-optimized structure of 2-CH_4^+ at the PBE0/BS2 level (21) and (B) two possible coordination modes for methane binding to a ligated (L_n) transition metal (M) center.



Scheme 1.

difference between the iridium(III) methyl hydride complex and the iridium(I) methane adduct, as well as the substantial binding energy of the alkane adduct, suggested the possibility of observing a long-lived σ -methane complex if the M^I oxidation state could be modestly stabilized relative to the M^{III} state.

The ease of reduction of rhodium relative to iridium motivated us to explore the analogous chemistry of the second row congener. The rhodium(I) methyl complex, (PONOP)RhCH₃ (**2-CH₃**), was prepared in a straightforward manner by reaction of methyl lithium with the corresponding halide precursor (21). Protonation of **2-CH₃** with $\text{H}[\text{B}(\text{Ar}^F)_4] \cdot (\text{Et}_2\text{O})_2$ in CDCl_2F solvent at -110°C yielded the rhodium(I) σ -methane complex, $[(\text{PONOP})\text{Rh}(\text{CH}_4)][\text{B}(\text{Ar}^F)_4]$ (**2-CH₄⁺**) (Scheme 1), which was characterized by a series of low-temperature NMR experiments (21). To facilitate spectroscopic characterization, we also prepared the ^{13}C labeled isotopologue, **2-¹³CH₄⁺**, in a similar fashion (21).

The ^1H coupled ^{13}C NMR spectrum of **2-¹³CH₄⁺** exhibits a quintet resonance at -41.7 parts per million (ppm) [coupling constant ($J_{\text{C-H}}$) = 124 Hz] (Fig. 3). This signal is shifted dramatically upfield with respect to **2-CH₃** and the iridium(I) methyl hydride cation, **1-(H)CH₃⁺**, which exhibit ^{13}C NMR signals at -21.8 and -20.6 ppm, respectively. The quintet multiplicity

of the signal indicates all four protons of the bound methane are in fast exchange. The observed $J_{\text{C-H}}$ of 124 Hz for **2-¹³CH₄⁺** is comparable to that of free methane in CDFCl_2 solution (125 Hz) (21) and substantially larger than the expected average coupling constant for a rapidly fluxional transition metal methyl hydride complex. For example, **1-(H)CH₃⁺** exhibits a quintet signal in the ^1H coupled ^{13}C NMR spectrum with an observed $J_{\text{C-H}}$ = 93 Hz under conditions where the Ir-H and Ir-CH₃ protons exchange rapidly (fig. S3). This observed coupling constant is as expected for the weighted average of a $^1J_{\text{C-H}}$ $\sim$ 120 Hz for the Ir-CH₃ group and a $^3J_{\text{C-H}}$ $<$ 5 Hz for the Ir-H and is substantially lower than that observed for the methane complex, **2-¹³CH₄⁺**.

Upon ^1H decoupling, the quintet signal in the ^{13}C NMR spectrum of **2-¹³CH₄⁺** collapsed to a singlet with a line width at half-height ($\nu_{1/2}$) of 6.7 Hz, nearly identical to that observed in the ^1H coupled ^{13}C NMR spectrum and substantially broadened with respect to the signal for free methane ($\nu_{1/2}$ = 1.8 Hz) (21). The width of the bound methane resonance may be attributed to unresolved coupling to the ^{103}Rh and ^{31}P nuclei. The absence of detectable coupling from these heteronuclei indicates a reduced interaction between the carbon of the methane ligand and the (PONOP)Rh^I fragment, particularly when contrasted to the coupling constants ($^1J_{\text{Rh-C}}$ =

25 Hz and $^2J_{\text{P-C}}$ = 11 Hz) observed for precursor **2-CH₃**.

The ^1H NMR spectrum of **2-CH₄⁺** at -110°C exhibited the number of resonances expected for a C_{2v} symmetric molecule with a broad doublet at -0.86 ppm ($J_{\text{Rh-H}}$ = 6.3 Hz) (21). Heteronuclear multiple-quantum coherence NMR and ^{13}C labeling (Fig. 3) experiments indicated that this proton resonance correlates to the carbon signal at -41.7 ppm, supporting its assignment to the bound methane ligand. The observation of a single broad resonance for the CH₄ adduct again suggests that all four hydrogens are in fast exchange. The observed rhodium-hydrogen coupling constant of 6.3 Hz is substantially higher than the corresponding coupling constant of 2.3 Hz for **2-CH₃**, consistent with an enhanced interaction between the metal and alkane hydrogens. Additionally, no detectable phosphorus-hydrogen coupling was observed, and selective decoupling of the ^{31}P resonance (206.5 ppm) did not result in an observable change in linewidth for the resonance at -0.86 ppm.

Further evidence for the assignment of the rhodium(I) σ -methane adduct was obtained from protonation of the (PONOP)RhCD₃ isotopologue with $\text{H}[\text{B}(\text{Ar}^F)_4] \cdot (\text{Et}_2\text{O})_2$. The resulting alkane complex, **2-CD₃H⁺**, displayed a slightly upfield shifted resonance at -1.02 ppm in the ^1H $\{^1\text{H}\}$ NMR spectrum at -110°C . Equilibrium isotope effects favor placing the C-D bonds in the tighter energy well (the terminal positions) and the C-H bond in the flatter energy well (the bond interacting with the metal center) (22). On the basis of an analogy with similar agostic complexes (6), the chemical shift of the hydrogen in the metal-bound site will exhibit a higher field ^1H NMR chemical shift than that for the hydrogen in the terminal site. Thus, the weighted average ^1H NMR chemical shift for the **2-CD₃H⁺** complex should appear upfield of that for the **2-CH₄⁺** complex, as is observed. Similar examples of isotopic perturbations of

chemical shifts are seen in partially deuterium-labeled fluxional alkyl agostic complexes (6).

To obtain further information about the coordination mode of the σ -methane ligand, we optimized the geometry of 2-CH_4^+ by using DFT, and the resulting coordination environment about the rhodium center is depicted in Fig. 4A (21). Calculations using the complete ligand framework showed an unsymmetrical interaction between rhodium and the methane ligand, with only one C-H bond coordinated to the metal ($\eta^2\text{-C,H}$; Fig. 4B). The $\eta^2\text{-C,H}$ coordination mode calculated for 2-CH_4^+ is comparable to that reported by Ball and co-workers for the interaction between the 16-valence electron Cp^*ReL_2 fragment and higher alkanes (vide supra) (15) and for other computational studies of methane complexes (23–26). A small barrier of less than 1 kcal/mol is computed for interchanging H_a and H_b and may originate from facile access to a symmetric $\eta^2\text{-H}_2$ coordination mode (Fig. 4B) by the 14-valence electron $(\text{PONOP})\text{Rh}^I$ moiety.

Computational studies suggest that the ground state for the methane adduct, 2-CH_4^+ , lies ~ 8 kcal/mol below the ground state for the unobserved methyl hydride complex, $[(\text{PONOP})\text{Rh}(\text{H})\text{CH}_3]^+ [2\text{-(H)CH}_3]^+$ (21). The rate of methane loss from 2-CH_4^+ was determined by ^{31}P NMR spectroscopy (20) and afforded an observed first-order rate constant of $1.4 \times 10^{-4} \pm 0.3 \times 10^{-4} \text{ s}^{-1}$ at -87°C , corresponding to $\Delta G^\ddagger = 14.5 \pm 0.4 \text{ kcal/mol}$ (27). The product of methane extrusion was tentatively assigned as the rhodium(I) solvated cation, $[(\text{PONOP})\text{Rh}(\text{CDFCl}_2)]^+ [\text{B}(\text{Ar}^F)_4]^-$. Free methane was detected concomitant with and roughly proportional to the formation of $[(\text{PONOP})\text{Rh}(\text{CDFCl}_2)]^+ [\text{B}(\text{Ar}^F)_4]^-$. The observed barrier to methane loss is consistent with previous experimental studies of methane reductive elimination/isotopic scrambling from platinum(II) centers, which predicted a lower limit for the methane dissociation enthalpy of 9 kcal/mol (28).

Protonation of a rhodium(I) methyl complex at low temperature has permitted the observation and full characterization by NMR spectroscopy of a relatively long-lived σ -methane complex in solution. This methane complex is the simplest alkane analog of the now classic σ -dihydrogen complexes first reported some 25 years ago (1). Just as these dihydrogen species provide insight into the early stages of metal-mediated cleavage of the H-H bond, the σ -methane complex 2-CH_4^+ furnishes details about the weak interaction of methane with a transition metal center before C-H bond scission. Of particular interest is that 2-CH_4^+ is isoelectronic with $\text{Pt(II)}\text{-}\sigma\text{-methane}$ complexes thought to be intermediates in Pt(II) -mediated Shilov-type oxidations of methane (29), the most thoroughly investigated systems for transition metal-catalyzed methane functionalization to date.

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Supporting Online Material

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Isolation of a C5-Deprotonated Imidazolium, a Crystalline "Abnormal" N-Heterocyclic Carbene

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The discovery two decades ago of metal-free stable carbenes, especially imidazol-2-ylidenes [N-heterocyclic carbenes (NHCs)], has led to numerous breakthroughs in organic and organometallic catalysis. More recently, a small range of complexes has been prepared in which alternative NHC isomers, namely imidazol-5-ylidenes (also termed abnormal NHCs or αNHCs , because the carbene center is no longer located between the two nitrogens), coordinate to a transition metal. Here we report the synthesis of a metal-free αNHC that is stable at room temperature, both in the solid state and in solution. Calculations show that the αNHC is more basic than its normal NHC isomer. Because the substituent at the carbon next to the carbene center is a nonbulky phenyl group, a variety of substitution patterns should be tolerated without precluding the isolation of the corresponding αNHC .

For decades, carbenes, which feature a neutral divalent carbon atom with two nonbonding electrons, were considered prototypical reactive intermediates (1). Today, thanks to the availability of stable carbenes (2, 3), these molecules, especially the so-called N-heterocyclic carbenes (NHCs) (4–6) (Fig. 1, top left), are recognized as versatile ligands for transition metal-based catalysts (7–10) and as metal-free organic catalysts in their own right (11–14). As expected, NHCs **I** usually bind metals via the

carbene center (C2) to give η^1 complexes **II**. However, in 2001, Crabtree and co-workers discovered that 2-pyridylmethylimidazolium salts react with $\text{IrH}_2(\text{PPh}_3)_2$ to give **1** with the imidazole ring bound

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the “wrong way,” at C5 and not at C2 (15, 16) (Fig. 1, center). Since that time, a few other complexes of type **IV** featuring the so-called abnormal

NHCs (*a*NHCs) (**III**) (17–20) as ligands have been prepared (21–23) (Fig. 1, top right). Experimental and theoretical data suggest that *a*NHCs **III** are

even stronger electron-donor ligands than are NHCs **I**. In line with these observations, initial catalytic screening of *a*NHC metal complexes **IV** reveals promising results for the activation of unreactive bonds such as C–H and H–H (24–26). As an example, an *a*NHC palladium complex has been reported to be an efficient catalyst in the Heck olefination of aryl bromides, whereas the corresponding NHC analog is virtually inactive under identical conditions (24).

Lassaletta and co-workers (27) have shown that the deprotonation of imidazo[1,5-*a*]pyridinium salts **2** leads to free NHC **3** that can be isolated (Fig. 1, bottom). In contrast, using C2-substituted precursors, such as **4**, Lassaletta *et al.* did not observe the corresponding free *a*NHC. However, by performing the deprotonation reaction in the presence of [Rh(COD)Cl]₂, they were able to isolate the corresponding *a*NHC complex **5**. Because calculations predicted that the parent *a*NHC **III** (where R is equal to H) is only about 17 kcal mol^{−1} higher in energy than its NHC isomer **I** (18), it seemed that free *a*NHC derivatives were reasonable synthetic targets. We report here the isolation of a metal-free member of this class of heterocyclic compound.

By analogy with the classical synthetic route used to prepare NHCs, we chose imidazolium salt **6** as a precursor to the desired *a*NHC **9** (Fig. 2). The p*K*_a (where *K*_a is the acid dissociation constant) for loss of the C5-bound proton in the parent imidazolium salt (~33) (28) was calculated to be nine units higher than that for loss of the C2-bound proton (29); we therefore replaced the C2 hydrogen with a phenyl group. To offer kinetic protection to the C5 position, we appended bulky 2,6-diisopropylphenyl (Dip) substituents at both nitrogen atoms, as well as a second phenyl group at C4. Imidazolium salts **6** with various counterions were prepared in good yields after slight modifications to known synthetic procedures (30–32). They were fully characterized by spectroscopic methods, with a single-crystal x-ray diffraction study carried out for the bromide salt **6** (Br[−]) (Fig. 3, left).

All attempts to deprotonate the imidazolium tetrafluoroborate salt **6** (BF₄[−]) failed. However, small anions such as Cl[−] and Br[−] are known to accelerate heterolytic C–H bond cleavage through hydrogen bonding, and this effect has been used with C2- and C5-unsubstituted imidazolium salts to favor metallation of C2 (with the more acidic proton) over C5 (33). We reasoned that with C2 protected in **6**, small anions should promote the desired deprotonation reaction at C5. Indeed, when **6** (HCl·Cl[−]) was treated with two equivalents (34) of a lithium base such as *n*-butyllithium (*n*BuLi) or lithium diisopropylamide (LDA), the proton nuclear magnetic resonance (¹H NMR) spectrum of the resulting product showed the disappearance of the singlet at 8.7 parts per million (ppm) arising from C5(H) of **6**. In the ¹³C NMR spectrum, the C5 carbon gives rise to a very broad resonance at 190 ppm, which is significantly downfield of the corresponding resonance for the precursor **6** (124 ppm). Although these data indicated that a deprotonation had occurred, the

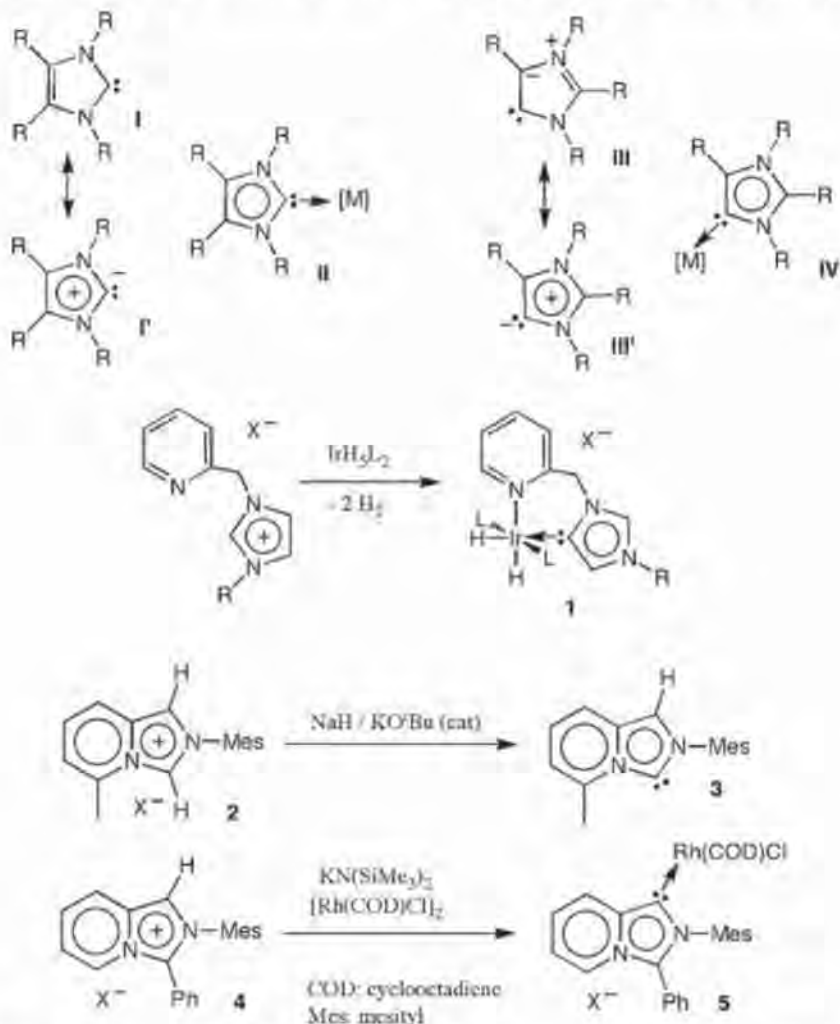


Fig. 1. (Top) Resonance structures for NHC (**I**) and *a*NHC (**III**), and their corresponding C2 and C5 metal complexes, **II** and **IV**, respectively. **(Center)** Synthesis of *a*NHC metal complex **1** by Crabtree and co-workers (15). **(Bottom)** Synthesis of metal-free NHC **3**, and *a*NHC metal complex **5** by Lassaletta and co-workers (27).

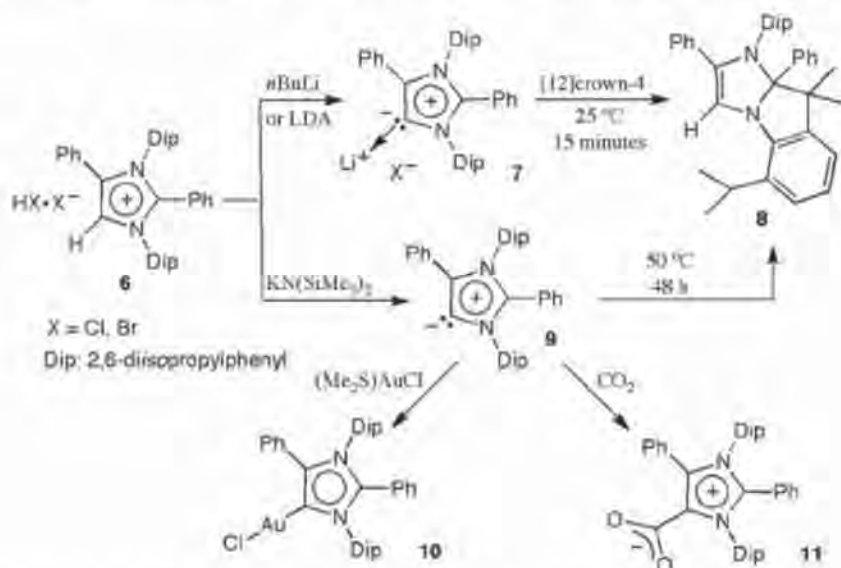


Fig. 2. Synthesis of *a*NHC lithium adduct **7, rearrangement product **8**, free *a*NHC **9**, and its ensuing gold(II) complex **10** and CO₂-adduct **11**.**

shape of the ^{13}C NMR signal, as well as the calculated chemical shift (32) for the C5 carbon of the free *a*NHC **9** (205 ppm), suggested that the new compound was the *a*NHC lithium **7**. Similar complexation has previously been observed for other singlet carbenes such as small NHCs (35) and bis(diisopropylamino)cyclopropenylidene (36, 37); in both cases, coordination of the lithium cation was apparent from the broadening and the upfield shift of the carbene ^{13}C NMR resonance. Subsequently, we sought to sequester the lithium cation through addition of excess [12]crown-4 to a diethylether solution of **7** (where X is Br). This treatment induced a clean rearrangement to generate **8**, which was isolated in 45% yield. This product formally results from the deprotonation of an isopropyl substituent of the Dip group by the carbene center of the *a*NHC lithium adduct **7**, followed by nucleophilic addition of the resulting benzyl anion to C2. However, calculations indicated that the rearrangement of **9** into **8** is exothermic by only 6.1 kcal mol $^{-1}$ and involves an activation barrier of 20.3 kcal mol $^{-1}$ for the proton transfer. Therefore, we hypothesized that the observed rearrangement was catalyzed by a

component of the (crown)LiBr system, and that the formation of **8** did not imply that the free *a*NHC **9** was too reactive to be isolated.

Sodium and potassium bases have proven more appropriate than lithium bases in generating free carbenes (35–37), because the corresponding carbon-heavy alkali metal bonds are more labile, which favors precipitation of the salt. When the deprotonation of imidazolium **6** ($\text{HX}\cdot\text{X}^-$, where X is Cl or Br) was performed with two equivalents (34) of potassium hexamethyldisilazide (KHMDs) in tetrahydrofuran, a clean reaction occurred, with the ^{13}C NMR spectrum of the resulting product showing a very sharp signal at 201.9 ppm. After the products were worked up, the free *a*NHC **9** was isolated as a green powder (480 mg, 68% yield), and single crystals were obtained by recrystallization from a dry hexane solution at -78°C (Fig. 3, right).

In the solid state, both the free *a*NHC **9** and the imidazolium salt **6** (Br^-) feature a fully planar ring (maximum deviation for N1–C2–N3–C4–C5–C21–C31–C41–C53 was 1.9 and 6.3 pm for **9** and **6**, respectively), confirming the delocalization of the π system. This electronic structure is corroborated by the values of the endocyclic C–N [6:

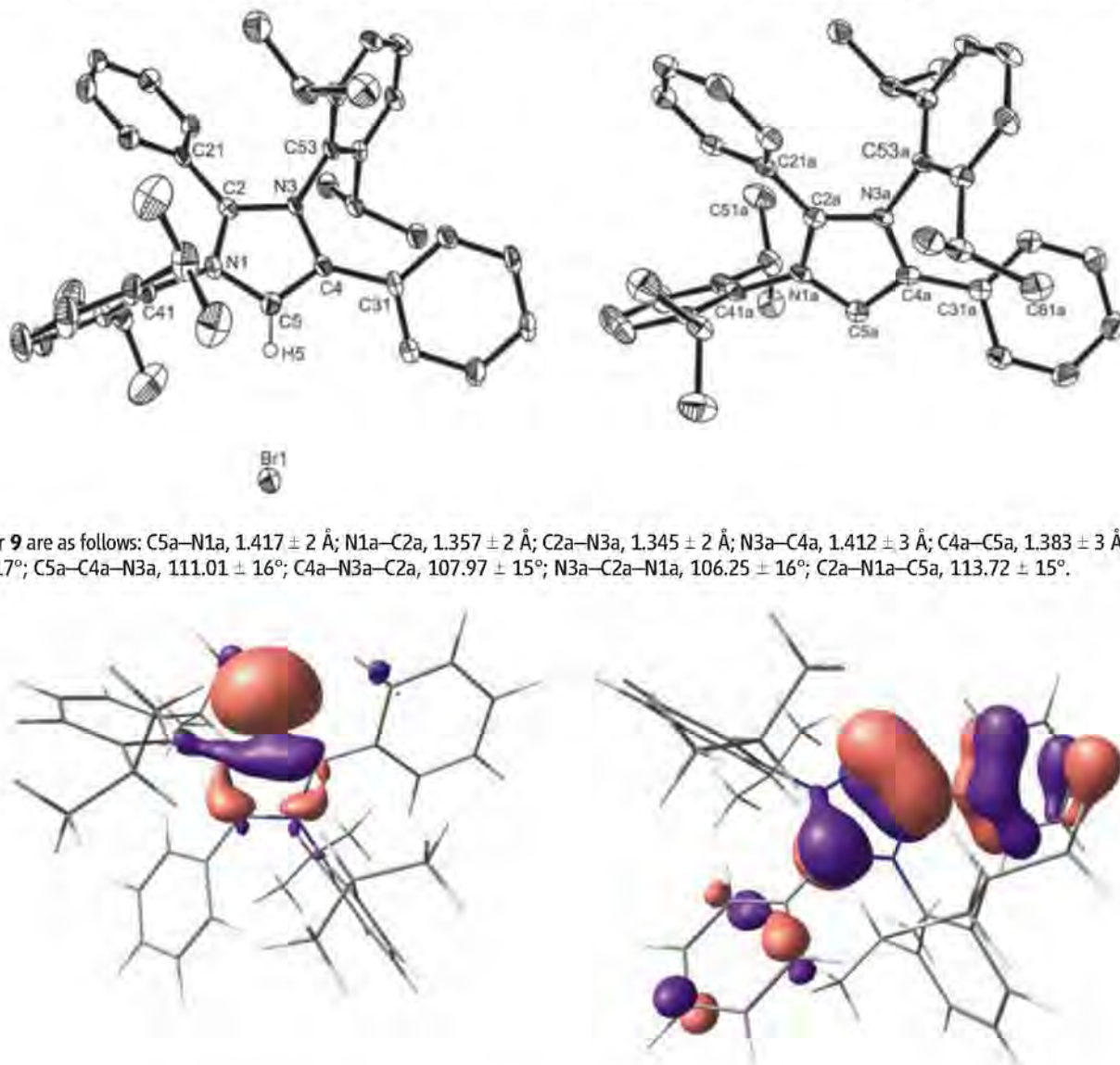
1.335 $\pm$ 5 to 1.409 $\pm$ 5; **9**: 1.354 $\pm$ 2 to 1.408 $\pm$ 3 Å] and C–C bond lengths [6: 1.351 $\pm$ 5, **9**: 1.385 $\pm$ 3 Å], which are halfway between those of single and double bonds. The carbene bond angle N1–C5–C4 for **9** [101.03 $\pm$ 17°] is more acute than the corresponding angle in the cationic precursor **6** [108.0 $\pm$ 3°]. This feature is consistent with increased *s* character of the σ lone-pair orbital on the carbene atom in **9** as compared with the C–H $^+$ bonding orbital in **6**. A similar relationship is observed in NHCs and their NHC(H^+) precursors (3, 4).

Calculations predict *a*NHC **9** to be 14.1 kcal mol $^{-1}$ less stable than its isomeric normal NHC with the phenyl group bonded to C2 instead of C2. Figure 4 shows the two highest occupied molecular orbitals (HOMOs) of **9**. The HOMO (–4.403 eV) is a σ -type lone-pair orbital at C5; the HOMO-1 (–4.879 eV) is a C5–C4 π -bonding orbital, which exhibits antibonding conjugation with the π orbital of the phenyl substituent at C4. These molecular orbitals are much higher in energy than those of the isomeric NHC (–5.000 and –5.279 eV, respectively), which indicates that

Fig. 3. Molecular views (50% thermal ellipsoids are shown) of imidazolium bromide **6** (Br^-) (left) and *a*NHC **9** (right) in the solid state (for clarity, H atoms are omitted, except for the ring hydrogen). Bond lengths and angles for **6** (Br^-) are as follows: C5–N1, 1.368 $\pm$ 4 Å; N1–C2, 1.334 $\pm$ 4 Å; C2–N3, 1.363 $\pm$ 4 Å; N3–C4, 1.408 $\pm$ 4 Å; C4–C5, 1.355 $\pm$ 5 Å; N1–C5–C4, 108.0 $\pm$ 3°; C5–C4–N3, 106.0 $\pm$ 3°; C4–N3–C2, 108.8 $\pm$ 3°; N3–C2–N1, 106.9 $\pm$ 3°; C2–N1–C5, 110.4 $\pm$ 3°.

Bond lengths and angles for **9** are as follows: C5a–N1a, 1.417 $\pm$ 2 Å; N1a–C2a, 1.357 $\pm$ 2 Å; C2a–N3a, 1.345 $\pm$ 2 Å; N3a–C4a, 1.412 $\pm$ 3 Å; C4a–C5a, 1.383 $\pm$ 3 Å; N1a–C5a–C4a, 101.03 $\pm$ 17°; C5a–C4a–N3a, 111.01 $\pm$ 16°; C4a–N3a–C2a, 107.97 $\pm$ 15°; N3a–C2a–N1a, 106.25 $\pm$ 16°; C2a–N1a–C5a, 113.72 $\pm$ 15°.

Fig. 4. Plot of the calculated two highest-lying occupied orbitals HOMO (left) and HOMO-1 (right) of the *a*NHC **9**.



*a*NHCs are more basic than NHCs. Indeed, calculations predict that the first (287.0 kcal mol⁻¹) and second (144.6 kcal mol⁻¹) proton affinity of *a*NHC **9** are significantly higher than those of normal NHCs (229.9 to 270.6 and 38.9 to 106.5 kcal mol⁻¹, respectively) (38).

Although abnormal NHC **9** is sensitive to air and quantitatively rearranges to **8** upon heating in benzene at 50°C for 48 hours, it is stable at room temperature for a few days both in the solid state (melting point: decomposition at 65°C) and in solution (39). The availability of stable *a*NHCs not only provides easy access to a variety of transition-metal complexes, but also allows for their use as organocatalysts. As a proof of concept (40–43), (*a*NHC)AuCl complex **10** and (*a*NHC)-CO₂ adduct **11** have been prepared in 79 and 95% isolated yields by simply reacting **9** with chloro(dimethylsulfide)gold(I) and CO₂, respectively (Fig. 2).

Because the substituent at the C4 of **9** is a nonbulky benzene ring, a variety of substitution patterns should be tolerated without precluding isolation of the corresponding *a*NHC. The substituent at C4 is in conjugation with the carbene center, which opens the possibility of substantially modulating the electronic character of the ring system.

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Supporting Online Material

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Materials and Methods
References

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Carbenes As Catalysts for Transformations of Organometallic Iron Complexes

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Compared with the enormous arsenal of catalysts used to produce organic compounds, complementary species that are able to mediate sophisticated organometallic transformations are virtually nonexistent. We found that stable N-heterocyclic carbenes (NHCs) can mediate unusual organometallic transformations in solution at room temperature. Depending on the choice of NHC initiator, stoichiometric or catalytic reactions of bis(cyclooctatetraene)iron [Fe(COT)₂] ensue. The stoichiometric reaction leads to the isolation of a previously unknown mixed-valent species, featuring distinct and directly bonded Fe(0) and Fe(II) centers. In the catalytic process, three iron atoms are fused to afford the tri-iron cluster Fe₃(COT)₃, which is a hydrocarbon analog of Dewar's classic Fe₃(CO)₁₂ complex. The key step in both of these processes is proposed to involve the NHC's ability to induce metal-metal bond formation. These NHC-mediated reactions provide a foundation on which to develop future organometallic transformations that are catalyzed by organic species.

Carbenes are compounds with a neutral divalent carbon atom that feature either two singly occupied nonbonding orbitals (a triplet state) or a lone pair and an accessible vacant orbital (a singlet state). With only six electrons in its valence shell, the carbene center defies the octet rule, and for many years, carbenes were considered to be prototypical reactive intermediates (1). The view that carbenes could exist only

as transient species was shattered in the late 1980s and early 1990s by the isolation of singlet phosphinosilyl (2) and N-heterocyclic carbenes (NHCs) (3), respectively, that were able to be bottled. These pioneering studies paved the way for the current revolution in carbene chemistry, which has rapidly developed over the past two decades (4–7). Although fundamental interest has contributed, the primary driving force for the explosion of research in this area is that NHCs and related species (8, 9) have great synthetic utility.

Acting as strong electron donors, such species readily bind other molecules (10–13). Two of the best-known exploitations of this property are the

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use of NHCs as ancillary ligands for transition-metal catalysts (4–7) and as organocatalysts in their own right (14). The former application often renders transition-metal species far more active and selective than classical phosphine-based systems, as exemplified by the current NHC-supported ruthenium olefin metathesis catalysts

(15, 16). The latter application, so-called organocatalytic carbene catalysis, is rooted in the pioneering studies of Breslow (17), which implicated transient NHCs as intermediates in the thiamine-promoted benzoin condensation reaction. The use of stable NHCs, particularly Enders' triazolylidenes (18), in place of thiamine has led

to highly efficient systems for the benzoin condensation and related reactions, as well as numerous distinct transformations (14, 19, 20). The unifying theme in these metal- and carbene-catalyzed approaches is that they both mediate organic transformations to produce organic molecules of potential interest, ranging from materials to pharmaceuticals.

What if it were possible to use NHCs in a different manner; namely, to catalyze organometallic transformations, resulting in metal-metal (M–M) bond formation? Organometallic compounds containing direct M–M bonds often display fascinating structures and play a key role in our understanding of the interactions between metallic elements (21–23). Certain complexes spontaneously form M–M bonded units, such as the metal carbonyls (24), in response to low coordination number. However, more often than not, the preparation of M–M bonded organometallic compounds requires the strategic stoichiometric reduction of the appropriate mononuclear complexes.

We report here that NHCs are capable of mediating sophisticated organometallic transformations. Depending on the choice of carbene initiator, we observed stoichiometric or catalytic processes, which ultimately produce unexpected organometallic complexes. We propose that the observed reactivity is imparted by the NHCs' ability to induce a key M–M bond-forming reaction.

There is a great need for environmentally friendly and inexpensive alternatives to traditional transition-metal catalysts, and so there has recently been a renaissance in iron-mediated reaction methodology (25–27). We entered this arena by targeting noncarbonyl, unoxidized iron complexes, bearing monodentate, stable carbene ligands. Such complexes are absent from the literature, and we had hoped that appending NHCs to Fe(0) would afford extremely reactive low-coordinate products. The most direct route to such complexes would be the addition of a carbene to an Fe(0) center, coordinated by labile ligands such as simple arenes or olefins. However, the reported synthetic protocols for such Fe(0) precursors are not practical on a large scale, because they require the vaporization of elemental iron and its co-condensation with the desired ligand (28, 29). A more convenient source of noncarbonyl Fe(0), albeit with a less

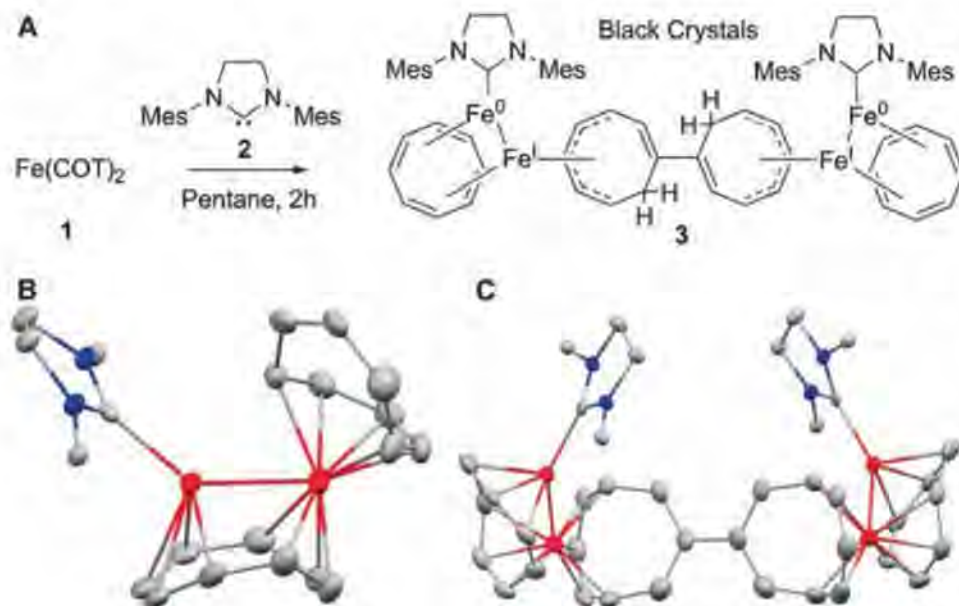


Fig. 1. (A) NHC 2 induces the transformation of $\text{Fe}(\text{COT})_2$ into the tetrametallic species 3, featuring two Fe–Fe bonded subunits. The solid-state molecular structure (displacement ellipsoids drawn at the 50% probability level) of 3 is shown in expanded view (B) of one of the two equivalent di-iron subunits, and in full (C). Red, Fe; gray, C; blue, N. Protons and Mes groups are omitted for clarity.

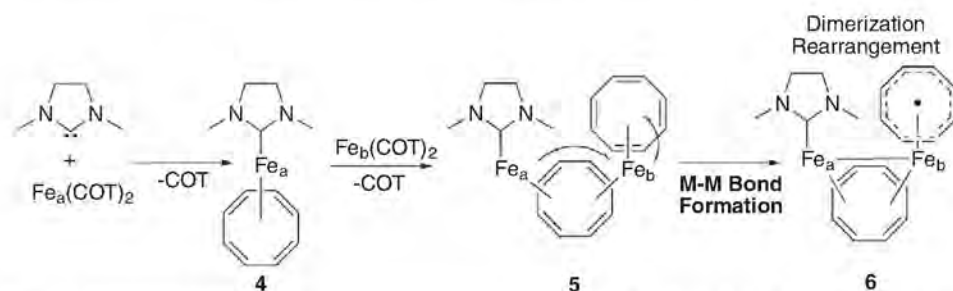


Fig. 2. Proposed pathway for the stoichiometric formation of 3, with a key NHC-induced M–M bond-forming step.

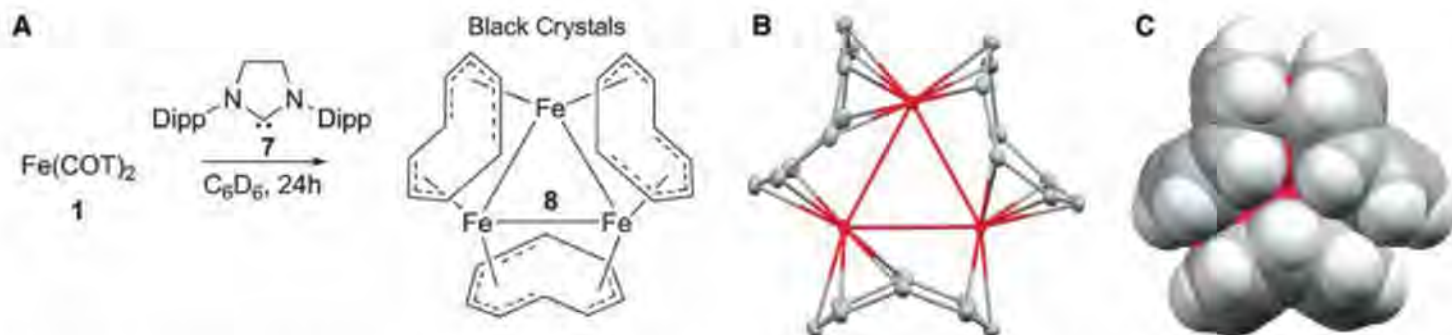


Fig. 3. (A) NHC 7 catalyzes the formation of the tri-iron cluster $\text{Fe}_3(\text{COT})_3$ 8. The solid-state molecular structure of star-shaped 8, rendered as balls and sticks (displacement ellipsoids drawn at the 50% probability level; protons omitted for clarity) (B), and in space-filling format (C) (red, Fe; gray, C; white, H) are shown.

labile hydrocarbon ligand, is bis(cyclooctatetraene) Fe(0), Fe(COT)₂ (Fig. 1A, complex **1**) (30). This readily available compound has excellent solubility in hydrocarbon solvents and has been reported to undergo the substitution of COT by certain phosphines (31).

We chose for a starting carbene ligand the commercially available *N,N*-bis(2,4,6-trimethylphenyl) (Mes)-substituted NHC (Fig. 1A, complex **2**). Thus, an unstirred pentane solution of Fe(COT)₂ **1** was treated with one equivalent of the NHC **2** at room temperature (Fig. 1) (32). Within minutes, small black crystals (Fig. 1A, complex **3**) began to precipitate from the blood-red solution and continued to form over 2 hours. If the reaction was performed using only half an equivalent of NHC **2**, an identical yield (79%) of **3** was obtained. Subsequent proton nuclear magnetic resonance (¹H NMR) analysis of the crystalline precipitate redissolved in [D₆]benzene revealed resonances ranging from +43.61 to -7.33 parts per million (ppm), which is suggestive of a paramagnetic species. As revealed through a single-crystal x-ray diffraction study of **3** (Fig. 1, B and C), a complicated series of reactions had occurred, resulting in the formation of a tetrametallic species featuring two equivalent di-iron regions. Based on our interpretation of the crystallographic data, the di-iron subunits feature distinctly coordinated Fe(0) and Fe(I) centers: The Fe(0) center is coordinated by the NHC ligand **2** and one half of a COT ring in an η⁴-coordination mode; the Fe(I) center shares the other half of this COT ring through η¹ binding and is also supported by a separate pentadienyl moiety in a COT-derived ring that connects the two di-iron subunits through a newly formed C-C bond. The close proximity of the mixed valent Fe(0)-Fe(I) centers (Fe-Fe distance, 2.508 Å) suggests an M-M single bond, which would give an overall 16- and 18-electron count at each metal center, respectively. Such mixed-valent Fe(0)-Fe(I) species are of special interest, because they are reminiscent of the elusive proposed intermediate (33) in the Fe-only hydrogenase (34) cycle.

How does the NHC induce the cascade of reactions that lead to the formation of **3**? We propo-

pose that, because the NHC remains coordinated to one of the iron centers in the di-iron subunit, the initial step is the substitution of a single COT ligand from Fe_a(COT)₂, to afford the highly reactive intermediate (NHC)Fe_a(COT) (Fig. 2, complex **4**). Subsequently, the reaction of **4** with Fe_b(COT)₂ produces the bimetallic intermediate **5**, where both iron atoms are held in close proximity by the bridging COT ligand. At this point the NHC-coordinated Fe_a attacks its stabilized neighbor Fe_b, forming an Fe-Fe single bond. In turn, Fe_b transfers a single electron to its nonbridging COT ligand, to afford a ligand-centered radical **6**, which, after dimerization and rearrangement, produces the product **3**.

To further investigate this unusual organometallic transformation, we attempted to prepare a stable version of the initially formed intermediate (NHC)Fe(COT) **4** by using the steric protection of the much bulkier *N,N*-bis(2,6-diisopropyl)phenyl (Dipp)-substituted NHC ligand (Fig. 3, ligand **7**). Thus, a [D₆]benzene solution of Fe(COT)₂ **1** was treated with one equivalent of the NHC **7** at room temperature (Fig. 3A) (32). Over a period of 24 hours, the unstirred blood-red solution became orange, and large black rhomboidal crystals (**8**) formed. The crystalline and pyrophoric precipitate **8**, which is nearly insoluble in common organic solvents at room temperature, could be dissolved sufficiently in [D₆]benzene for ¹H NMR analysis. In solution, **8** exhibits a single, slightly broad (width = 0.40 ppm) paramagnetically shifted resonance at -3.15 ppm, which suggests the formation of a new species that contains iron and at least one coordinated and fluxional COT ligand. Elemental analysis of **8** indicated a compound with an atomic distribution corresponding to a 1:1 ratio of iron and cyclooctatetraene, Fe(COT).

To ascertain the molecular formula and connectivity of **8**, a single-crystal x-ray diffraction study was undertaken. The crystal structure reveals that three Fe(COT) units have been fused to form the tri-iron cluster Fe₃(COT)₃ **8** (Fig. 3, B and C). The three iron atoms of **8** form an almost perfect equilateral triangle (angles centered at Fe1 =

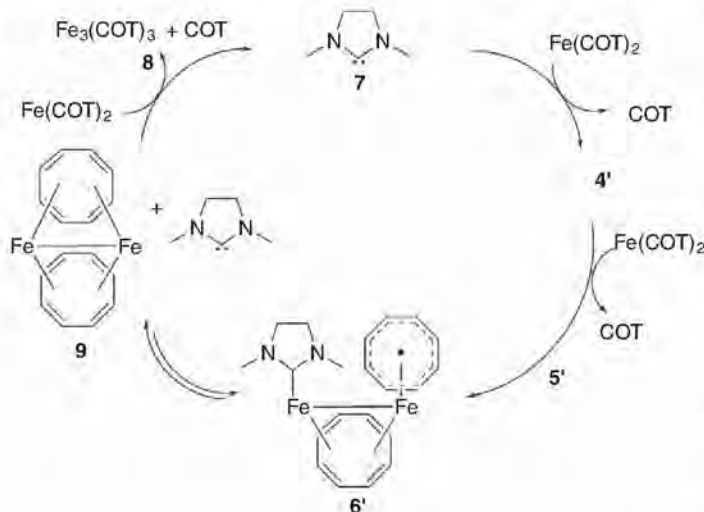
59.67°, Fe2 = 60.15°, and Fe3 = 60.18°), with an average Fe-Fe distance of 2.824 Å (Fe1-Fe2 = 2.829 Å, Fe1-Fe3 = 2.815 Å, and Fe2-Fe3 = 2.830 Å). The proximity of the iron atoms of **8** suggests direct M-M single bonds (the range for reported Fe-Fe single bonds is 2.20 to 3.12 Å) (35), which would give this molecule the expected closed-shell configuration for 48-electron trinuclear clusters (24). Each of the three bridging COT ligands adopts coordination modes approaching η³ and η⁵, crowning the iron triangle to form a six-pointed star. A space-filling model of **8** (Fig. 3C) shows that the iron core is completely enveloped by the COT ligands, forming a kinetically protective hydrocarbon shell. The unusual hapticity of the COT ligands suggests that they have a substantial amount of π-allyl and pentadienyl character, which probably results from partial metal-to-ligand electron transfer. However, absent a detailed experimental and computational study of the electronic structure of **8**, we are hesitant to definitively assign the oxidation state of the iron atoms.

Complex **8** is a hydrocarbon analog of the classic tri-iron cluster Fe₃(CO)₁₀(μ-CO)₂, which was discovered over 100 years ago by Dewar and Jones as the first cyclic organometallic cluster compound (36). Moreover, Fe₃(COT)₃ is the only homoleptic trimetallic cluster among group-8 transition metals to bear all-organic, noncarbonyl ligands. The elusiveness of this coordination environment in zero-valent d⁸ metal complexes is largely caused by the difficulty of stabilizing such electron-rich clusters without the aid of the strong π-accepting properties of carbonyl ligands.

The observed formation of Fe₃(COT)₃ **8** is surprising, because Fe(COT)₂ **1** has been reported to be indefinitely stable at room temperature in hydrocarbon solvents (37); the reaction must be attributed to the presence of the NHC **7**. To explore the catalytic efficiency, we added 10 mole percent of NHC **7** to a benzene solution of Fe(COT)₂ **1**, and over a 24-hour period, Fe₃(COT)₃ **8** slowly crystallized from the reaction mixture in 67% yield, corresponding to a turnover number (TON) of 6.7. If the reaction is conducted at 45°C over the same time period, the yield increases to 95% (TON = 9.5). From control experiments, which were conducted using identical experimental parameters (minus the NHC **7**), we observed that it is possible to detect small amounts of Fe₃(COT)₃ under forcing conditions (100°C, C₆H₆, 24 hours) without a catalyst. However, the resultant precipitate is contaminated by copious metallic iron that hampers the purification of **8**.

How does the NHC catalyze the formation of **8**? We believe that the pathway shares common intermediates with the formation of **3**, but the outcomes of these reactions are different based on steric effects of the NHC initiators. We propose that with the larger NHC **7**, the first several steps of these reactions are the same, namely COT displacement (Fig. 4, complex **4'**), combination with Fe(COT)₂ **5'**, and Fe-Fe bond for-

Fig. 4. Proposed catalytic cycle for the formation of **8** mediated by NHC **7** (Dipp groups are omitted for clarity).



mation to afford the ligand-centered radical intermediate **6'** (Fig. 4). In the case of the smaller NHC **2**, because of a lack of kinetic protection as well as strong binding of the carbene ligand, the dimerization process of **6** proceeds and the mixed valent Fe(0)–Fe(I) species is formed stoichiometrically (Fig. 2). However, when the more sterically demanding NHC **7** interacts with the iron center, the dimerization process is blocked, and the unstable species **6'** extrudes the NHC ligand. The ensuing Fe₂(COT)₂ species rapidly combines with Fe(COT)₂ to form the tri-iron cluster. Of course, it is possible that the NHC may not dissociate until the third Fe(COT) fragment is added to **6'**. There is ample precedent in the literature for NHC lability, particularly in zero-valent, late transition-metal complexes (38). Moreover, steric hindrance at the metal center reportedly facilitates this process (39).

Once thought of only as laboratory curiosities, stable carbenes are now widely recognized as indispensable tools for organic synthesis. The NHC-mediated reactions we explored provide a foundation on which to develop future organometallic transformations catalyzed by NHCs, as well as other small organic species.

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Detection of Adsorbed Water and Hydroxyl on the Moon

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Data from the Visual and Infrared Mapping Spectrometer (VIMS) on Cassini during its flyby of the Moon in 1999 show a broad absorption at 3 micrometers due to adsorbed water and near 2.8 micrometers attributed to hydroxyl in the sunlit surface on the Moon. The amounts of water indicated in the spectra depend on the type of mixing and the grain sizes in the rocks and soils but could be 10 to 1000 parts per million and locally higher. Water in the polar regions may be water that has migrated to the colder environments there. Trace hydroxyl is observed in the anorthositic highlands at lower latitudes.

The quest to find water on the Moon has been underway since the prediction of ice in the permanently shadowed craters in the polar regions (1, 2). Water is a vital resource needed by a human colony and played a fundamental role in planetary evolution (3). Spectroscopy is a tool that can be used to detect water and hydroxyl in the optical surface (the top few mm) using OH and H₂O absorptions near 1.5, 2, and 3 μ m.

It is thought that the Moon formed by a collision between Earth and a Mars-sized body (4) about 4.4×10^9 years ago. The impact and the

accretion of the debris heated the early Moon, creating an extensive lunar magma ocean (5, 6). These events are thought to have resulted in the loss of almost all volatiles in the Moon, and indeed evidence from Apollo and Luna samples indicate that lunar materials are deficient in volatiles compared with Earth (3). However, 20 to 45 parts per million (ppm) water occurs in some Apollo lunar glasses (7).

In addition, neutron spectrometer data from Lunar Prospector (LP) (8–10) showed that hydrogen is present in the lunar polar regions. I used data from the Visual and Infrared Mapping Spectrometer (VIMS) (11) on Cassini, which flew by the Moon on 19 August 1999, to map the dis-

tribution of water (Fig. 1). VIMS obtained 11 full and 2 partial image cubes with a spatial resolution of about 175 km per pixel and spectral coverage over the 0.35 to 5 μ m with some gaps due to sensor saturation [supporting online material (SOM) text and fig. S1].

Where the moon is warmed by solar radiation, thermal emission contributes to the observed signal, $(I + T)/F$, where I is measured radiance plus thermal emission T and πF equals solar radiance. The thermal emission component and emissivity effects can mask weak water absorptions, but not strong ones, nor sharper absorptions due to hydroxyl. Thermal emission was computed and removed from lunar spectra (Fig. 2A) by using a thermal model (12, 13) (SOM text and figs. S2 and S3), which included estimating the emissivity from reflectance by using Kirchhoff's law. Thermal-removed lunar spectra show water and hydroxyl absorptions in many but not all locations on the lunar disk (Figs. 1, E and F, and 2, A and B). The linear, positive-sloping spectral shape (Fig. 2A) is characteristic of the spectral signature of nanophase iron (14).

The lunar absorptions near 3 μ m are characteristic of an O–H stretch fundamental in the H₂O molecule commonly seen in spectra of materials with adsorbed water or hydroxyl-bearing materials (Fig. 2, C and D) (15). The VIMS broad 3- μ m absorption was strongest in the south lunar

Fig. 1. Cassini VIMS observations of the Moon on 19 August 1999. The VIMS flyby view was south of the lunar equator. (A) VIMS 2.4- μm apparent reflectance. (B) Cassini Imaging Science Subsystem image obtained during the flyby. The yellow bars indicated the equator position. The yellow cross indicates latitude 0, longitude 0. (C) Locations of VIMS spectra in Fig. 2A in the 2.4- μm apparent reflectance image. (D) VIMS-derived temperatures. Maps of (E) 3- μm absorption strength (blue) and (F) 2.8- μm OH strength (orange and green). (G) Hydrogen map from LP (7–9) masked to give a similar view as the VIMS observation.

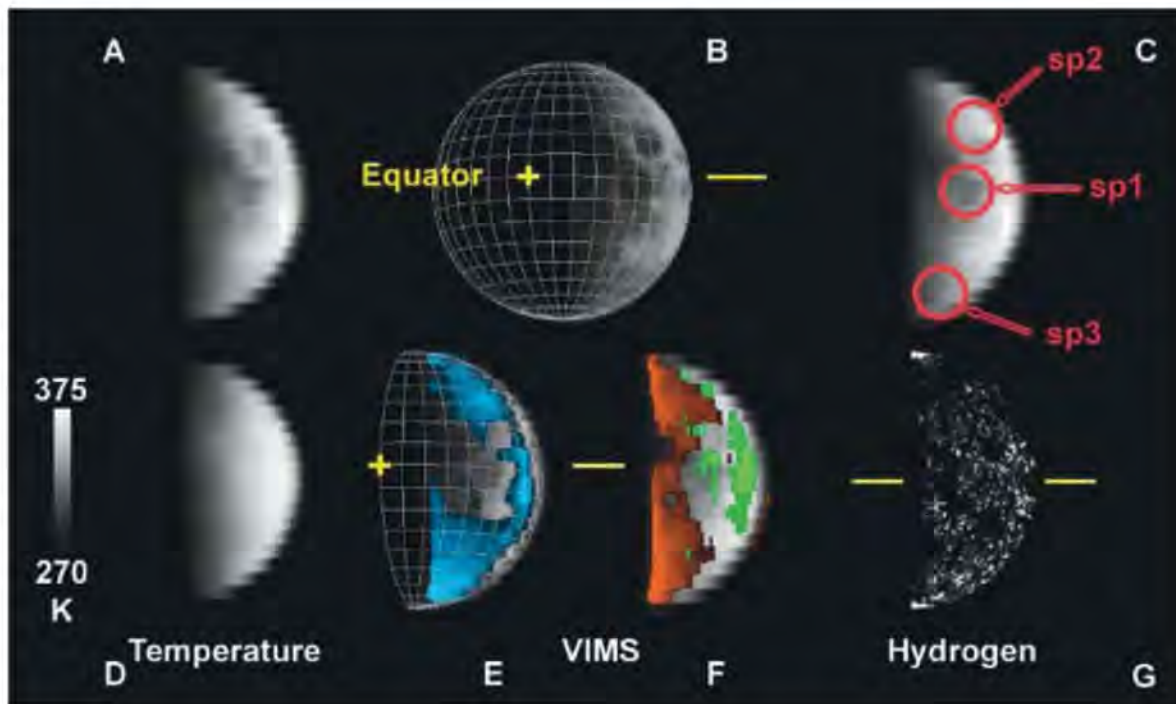
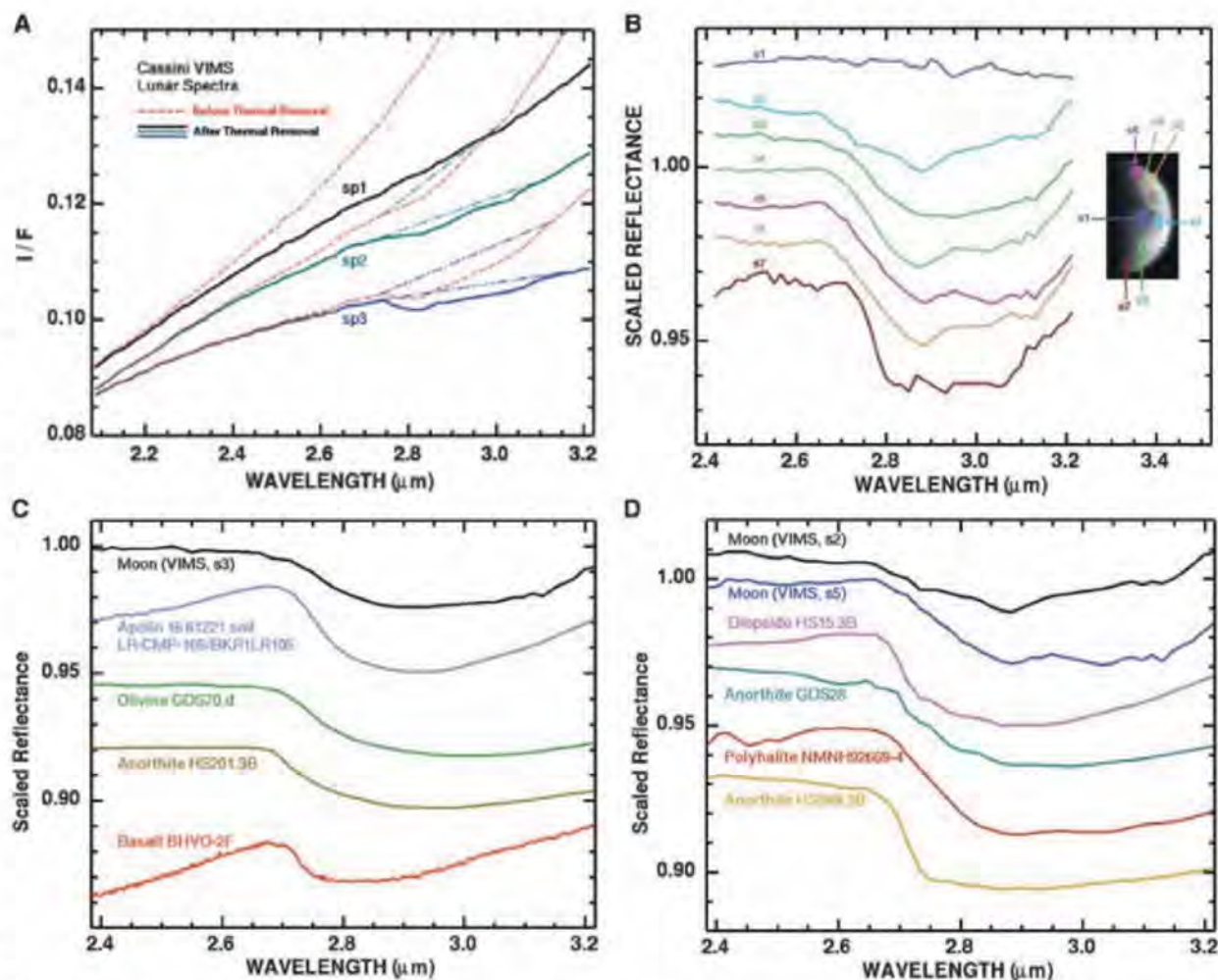


Fig. 2. (A) Average VIMS spectra for the three regions indicated in Fig. 1C. The spectra before thermal emission are shown as dashed red lines. Spectrum sp1 is dominated by maria and shows no water absorption. Spectrum sp2 is dominated by highlands and shows a weak absorption with a minimum near 2.8 μm , characteristic of hydroxyl. Spectrum sp3 includes the south polar region and indicates both a broad absorption from about 2.8 to 3.1 μm , characteristic of trace adsorbed water, as well as a stronger 2.8- μm absorption, characteristic of hydroxyl. The water and hydroxyl absorptions are also seen in the sp2 and sp3 spectra with thermal emission. The colored dash-dot lines are continua that illustrate the 2.8- and 3- μm absorptions. For ease of comparison, the spectra were scaled by 0.8 (sp1), 0.565 (sp2), and 1.0 (sp3). (B) Continuum-removed spectra showing varying 3- μm absorptions. The green areas in Fig. 1F have spectral shapes like that in spectrum s2; the orange areas have an ~ 2.85 minimum like those in s5 to s7. (C) Spectrum s3 (top), from (B), is shown compared with laboratory spectra of minerals, a basalt, and a lunar soil. (D) VIMS lunar spectra s2 and s5, from (B), show broad absorption because of water with sharper absorptions attributed to hydroxyl. Spectra of minerals (11) show similar structure.



The olivine and anorthite are from (11), the basalt was measured for this study in (B), and the Apollo 16 soil is from the RELAB spectral library, www.planetary.brown.edu/rehab. (D) VIMS lunar spectra s2 and s5, from (B), show broad absorption because of water with sharper absorptions attributed to hydroxyl. Spectra of minerals (11) show similar structure.

polar region and just north of Mare Crisium (Figs. 1E and 2B, spectra s6 and s7). Narrower absorptions, characteristic of hydroxyl fundamentals near 2.7 to 2.9 μm , are mapped in both the polar regions and in lunar highlands (Figs. 1F and 2B). The hydroxyl absorption mapped strongest in the polar regions and weaker but present at lower latitudes and along the lunar terminator (Fig. 1F). The lunar south polar region was tilted toward Cassini during the flyby, thus showing better coverage of that pole.

The host minerals for the water and hydroxyl are difficult to determine because the adsorbed water absorptions are not particularly unique and the 0.016- μm bandpass of the VIMS instrument was low. The position of the 3- μm absorption is consistent with ice as well as adsorbed water (Figs. 2, C and D, and 3), but ice is not stable in sunlight on the lunar surface. The water must be adsorbed or trapped in glass or in minerals. Adsorbed water has a wide range of wavelength positions and shapes that depend on the hydrogen bonding (16). The absorption minimum near 2.9 μm indicates that the water is strongly hydrogen bonded, which is also consistent with the harsh lunar environment, where only strongly bonded water might survive at the surface. Several minerals, including altered anorthite and pyroxenes, have hydroxyl fundamentals near 2.8 μm (15) and show spectral structure consistent with the lunar spectra.

Radiative transfer models (17) of typical lunar soil show that a water abundance of about 1000 ppm could produce a 2% absorption in VIMS spectra at 3 μm (Fig. 3). The strength of the absorption in the lunar spectrum sp3 in Fig. 2A is about 3%. A molecular mixture, where the water molecules are uniformly mixed in the lunar rocks or soils, has the highest sensitivity. For a typical grain size of 25 μm , models for a molecular mixture indicate that a 3% absorption would require about 10 ppm water if the reflectance were 40% (Fig. 3). For lower reflectances, higher water abundances are indicated. If the water was attached to some minerals and not others, and those minerals were in an intimate mixture, 3%

absorption would require 1000 ppm water at 40% reflectance. Laboratory dehydration experiments on basalt (SOM text) (fig. S4) show that a water content of 2500 ppm water at 18% reflectance yields a 9% absorption depth, so a 3% absorption would correspond to about 800 ppm, assuming a linear trend. The VIMS spectra are consistent with a water content of the sunlit lunar surface of 10 to 1000 ppm.

The 3- μm absorption detected by VIMS is confirmed by the Moon Mineralogy Mapper (M^3) on Chandrayaan-1 (18) and Deep Impact (DI) (19). DI confirms that the water absorption extends to low latitudes, but M^3 only shows absorption in the polar region because the limited spectral range makes low water amounts difficult to map. Both VIMS and DI data indicate stronger absorption near the lunar terminator, and Sunshine *et al.* (19) attribute this as evidence for movement of water with the diurnal cycle. However, viewing geometry might account for some or all of this apparent variability (SOM text and figs. S5 and S6).

LP (8–10) found relatively high concentrations of hydrogen at both lunar poles and smaller abundances at lower latitudes (Fig. 1F). The water detected by VIMS as well as M^3 and DI covers a larger area in the polar region than indicated by the LP hydrogen data. There is a correlation of the LP hydrogen data (Fig. 1G) and the VIMS absorptions (Fig. 1, E and F). Both VIMS and LP data indicate that the maria have low water content. The LP data represent a signal up to 1-m depth, whereas the VIMS data are sensitive to water and OH in the top millimeter or so of the surface. Water at the surface is more susceptible to destruction or escape than the buried hydrogen; thus, different patterns might be expected. The polar water seen in the VIMS data could be a thin surface effect from water migrating from low latitudes to the colder polar regions. Feldman *et al.* (8) argued against a surface deposit because it is not indicated by the fast neutron data. However, the neutron data are not sensitive to a low-abundance, very thin (mm) surface layer [see figure 3 in (8)], the depth probed by the VIMS data. This may indicate that the water and OH observed

in the VIMS data are not simply from a surface deposit in the polar regions but extend beneath the surface, and possibly have been mixed by impact gardening. At latitudes around the equator, VIMS detects some but not widespread water; however, hydroxyl is detected at all latitudes. The LP neutron data show high absorption near the equator (Fig. 1G) and may be indicating buried hydrogen that is too deep for VIMS to have detected.

About 10^{13} kg of water has been delivered to the lunar surface by comets over the past 2 billion years, or about 0.5 kg/m^2 (20). That amount distributed uniformly in the top millimeter of the surface would be about 50% abundance. Impact gardening would bury and mix that water in the top couple of meters, diluting the average abundance to about 500 ppm. The water that is detected by VIMS could be indicating the presence of that ancient water. Solar wind implanted protons could interact with oxygen-containing minerals and glasses in the lunar surface, creating H_2O and OH (21) species. Some of the signature reported here might originate from the solar wind protons. Regardless of its origin, water is found on the lunar surface in areas previously thought to have been depleted in volatiles.

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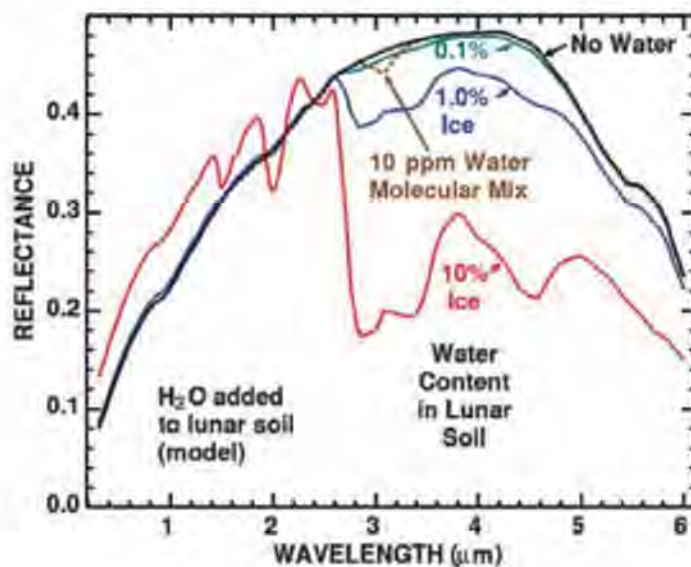
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Fig. 3. Radiative transfer models of water and hydroxyl-bearing minerals in different conditions and amounts on a model of Apollo 16 soil (containing no water or hydroxyl absorptions). Different scattering conditions and water abundances change the strength of the 3- μm water absorption.



Temporal and Spatial Variability of Lunar Hydration As Observed by the Deep Impact Spacecraft

Jessica M. Sunshine,^{1*} Tony L. Farnham,¹ Lori M. Feaga,¹ Olivier Groussin,² Frédéric Merlin,¹ Ralph E. Milliken,³ Michael F. A'Hearn¹

The Moon is generally anhydrous, yet the Deep Impact spacecraft found the entire surface to be hydrated during some portions of the day. Hydroxyl (OH) and water (H₂O) absorptions in the near infrared were strongest near the North Pole and are consistent with <0.5 weight percent H₂O. Hydration varied with temperature, rather than cumulative solar radiation, but no inherent absorptivity differences with composition were observed. However, comparisons between data collected 1 week (a quarter lunar day) apart show a dynamic process with diurnal changes in hydration that were greater for mare basalts (~70%) than for highlands (~50%). This hydration loss and return to a steady state occurred entirely between local morning and evening, requiring a ready daytime source of water-group ions, which is consistent with a solar wind origin.

EPOXI, NASA's extended mission for the Deep Impact spacecraft, is targeted to fly by comet 103P/Hartley 2 in November 2010. En route to the comet, the spacecraft has had numerous close approaches to the Earth-Moon system and has observed the Moon as a calibration source, particularly for the 1.05- to 4.5- μ m near-infrared spectrometer (1). The Moon Mineralogy Mapper spectrometer team (M³, on-board India's Chandrayaan-1 spacecraft) learned of our planned June 2009 calibrations and, given our spectral range, requested confirmation of their results (2, 3). We observed the Moon twice in June 2009, 1 week (one-quarter of a lunar day) apart, when the spacecraft was over the northern polar regions. Nearside equatorial regions were also observed in December 2007 (table S1). Together, these data enable us to explore the distribution of surficial OH/H₂O as a function of temperature, latitude, time of day, and composition.

The spectral range of the Deep Impact High-Resolution Instrument-infrared spectrometer (HRI-IR) includes the entire span of the broad 3- μ m hydration feature, while independently constraining thermal emissions that dominate at 4 to 4.5 μ m (4). In our analyses, we removed these thermal contributions (5) to reveal the full shape and structure of the hydration feature from 2.7 to 3.6 μ m (Fig. 1). The observed hydration features show a maximum absorption at 2.81 μ m, consistent with stretching vibrations of surface or structural hydroxyls (6, 7). A local minimum was also observed at ~2.95 μ m, and in some spectra an additional feature was present at ~3.14 μ m, which is commonly observed for hydrated minerals (6, 8, 9) and on Mars (10–12). The 2.95- μ m absorption can be attributed to asym-

metric and symmetric stretching modes of the H₂O molecule (6) or to OH bonds, depending on the bonding cations or bond energies. The first overtone of the H₂O bending vibration commonly occurs near 3.1 μ m and would be diagnostic of

H₂O. However, because of calibration uncertainties, this absorption is not distinctly resolved in our data. The overall shape of this lunar hydration feature is similar to hydration features observed on Mars (11, 12) and on certain classes of asteroids (13), both of which are attributed to OH-and/or H₂O-bearing phases.

These spectra were obtained as part of our calibration measurements by rapidly scanning the spectrometer slit lengthwise across the Moon such that every pixel along the slit crossed the lunar disk along the same chord. This resulted in a spectral average of all areas along that chord. These calibration measurements were obtained in full-resolution mode with 1024 spectral channels [versus the nominal 512 (1)], and each was acquired multiple times. Thus, these data have the highest signal-to-noise ratio and the highest spectral resolution of all our lunar observations. Three different lunar chords were measured (Fig. 1). The first chord (purple in Fig. 1A) was obtained on 29 December 2007 and lies on the evening side of the lunar disk, roughly parallel to the equator at 6°S to 11°S. The other two chords were obtained on 2 June 2009 when the spacecraft

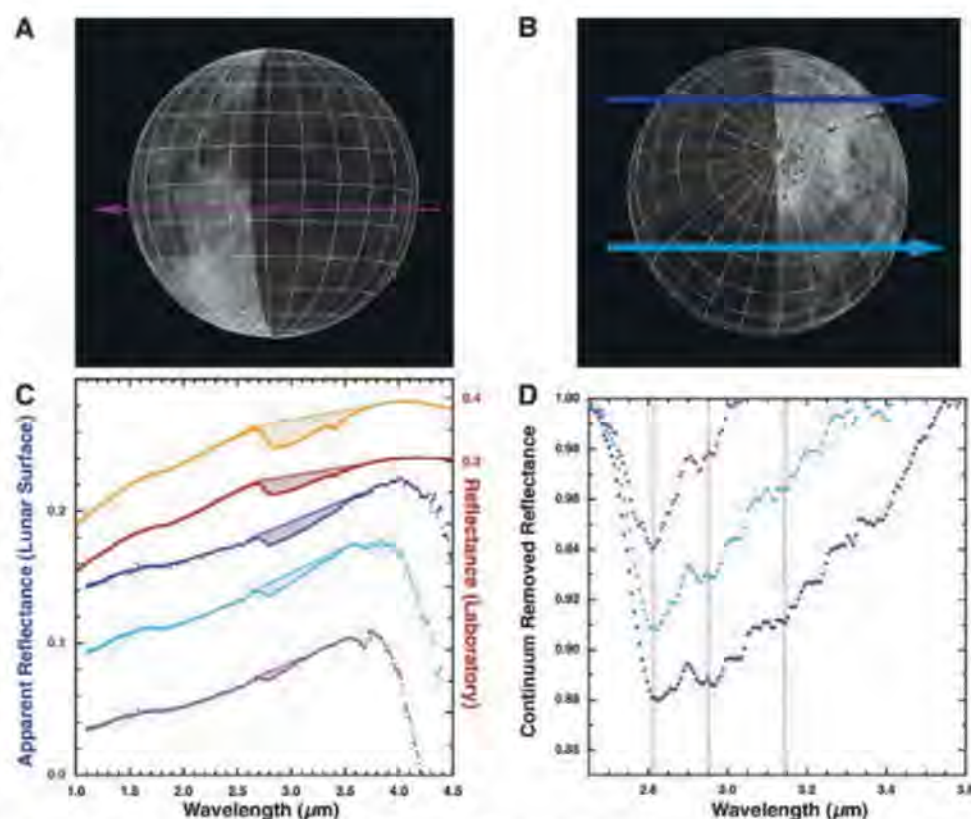


Fig. 1. High signal-to-noise reflectance spectra of the average response along a chord of the Moon acquired as Deep Impact scanned rapidly across the Moon. (A) Location of the equatorial chord (purple arrow) acquired on 29 December 2007 over a 750-nm Clementine basemap (15° grid). Arrow width indicates size of spectrometer slit. (B) Locations of two chords acquired on 2 June 2009 at mid-latitudes toward the morning (cyan arrow) and evening (blue arrow) sides. (C) Spectra of the three chords as compared to laboratory data of two lunar soils (14259, red; 62231, orange). Continua over the 3- μ m region (dashed) reveal absorptions due to hydration (shaded regions) similar to hydration features in the laboratory data (some, if not all, of which is terrestrial in origin). (D) Continuum-removed spectra of the 3- μ m regions of the Deep Impact chord spectra. The three major OH and H₂O absorptions near 2.8, 2.95, and 3.14 μ m are indicated (dotted red lines).

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was looking down on the northern hemisphere (blue and cyan in Fig. 1B) and cover mid-latitude regions ($\sim 20^\circ\text{N}$ to 60°N), with one on the morning side and the other on the evening side.

The three chord-averaged spectra all show well-defined absorption features in the $3\text{-}\mu\text{m}$ region, with minima at $\sim 2.81\text{ }\mu\text{m}$ and asymmetric

increases in reflectance toward longer wavelengths consistent with OH and/or H_2O as seen in laboratory spectra of lunar samples (in which at least some of the hydration is terrestrial in origin). The strongest absorption occurs in the mid-latitude evening chord (blue) from June 2009. The mid-latitude morning chord (cyan) is weaker,

but still relatively strong, whereas the December 2007 equatorial chord (purple) has the weakest absorption. As illustrated in Figs. 1, C and D, we fit a continuum across the feature (near 2.6 and $3.6\text{ }\mu\text{m}$) such that the depth of the $2.8\text{-}\mu\text{m}$ OH absorption and the equivalent width (or integrated band depth) from 2.6 to $3.6\text{ }\mu\text{m}$ can be measured (14). This results in $2.8\text{-}\mu\text{m}$ band depths of 12, 9, and 6% and equivalent widths of 0.06, 0.03, and $0.01\text{ }\mu\text{m}$ for the 2 June evening, morning, and December 2007 chords, respectively.

In addition to the integrated chord measurements, we collected three sets of spatially resolved imaging scans of the lunar surface at 512 wavelengths across the spectral range of the instrument. These scans were acquired by moving the spacecraft in a direction perpendicular to the slit of the spectrometer at a rate of one slit width per integration. To minimize saturation, we imaged the Moon using the 64 central pixels along the slit, which are covered by an antisaturation filter with the shortest possible integration time [0.72 s (1)]. A small area along the equator was imaged in December 2007, and the northern polar regions were scanned on both 2 June and 9 June 2009. Due to rotation of the Moon, the 90° of longitude that overlap between the June data sets are observed at two different local times of day (Fig. 2). The thermal contribution was removed from these data with the same method used for the chords (5), to produce temperature maps (Fig. 2, C and G) and, after subsequent removal of the spectral continuum over the $3\text{-}\mu\text{m}$ absorption, band depth maps (Fig. 2, D and H).

In the June 2009 polar data, hydration is detected in all pixels and thus at all latitudes greater than 10°N . The band depths also exhibit a clear dependence on temperature (i.e., insolation) and therefore on distance from the subsolar point (Fig. 2, D and H). Polar regions above 70°N have only minimal differences in band depth and, independent of local composition, exhibit the strongest absorptions. However, band strength is not solely dependent on latitude. All regions along the terminator (both morning and evening) were relatively cold ($\sim 280\text{ K}$) and contained similar OH and H_2O abundances, yet they span latitudes from 10°N to 90°N . Thus, spatial and temporal variations in volatile abundance appear to be more strongly controlled by the instantaneous, rather than cumulative, solar insolation.

The imaging scan acquired in December 2007 contains 64 by 64 pixels straddling the equator on the central nearside ($\sim 11^\circ\text{S}$ to $\sim 10^\circ\text{N}$) (Fig. 3). The warmer (sunward) third of the region lacked $3\text{-}\mu\text{m}$ absorptions and thus contained no detectable hydration. However, a weak $3\text{-}\mu\text{m}$ feature was evident toward the evening terminator (beginning at solar incidence angles of $>65^\circ$; $<\sim 330\text{ K}$) with a $2.8\text{-}\mu\text{m}$ band depth of $\sim 4\%$. These magnitudes are consistent with the chord measurement acquired contemporaneously at similar latitudes (Fig. 1). As with the polar observations, this equatorial scan indicates that instantaneous temperature, not lati-

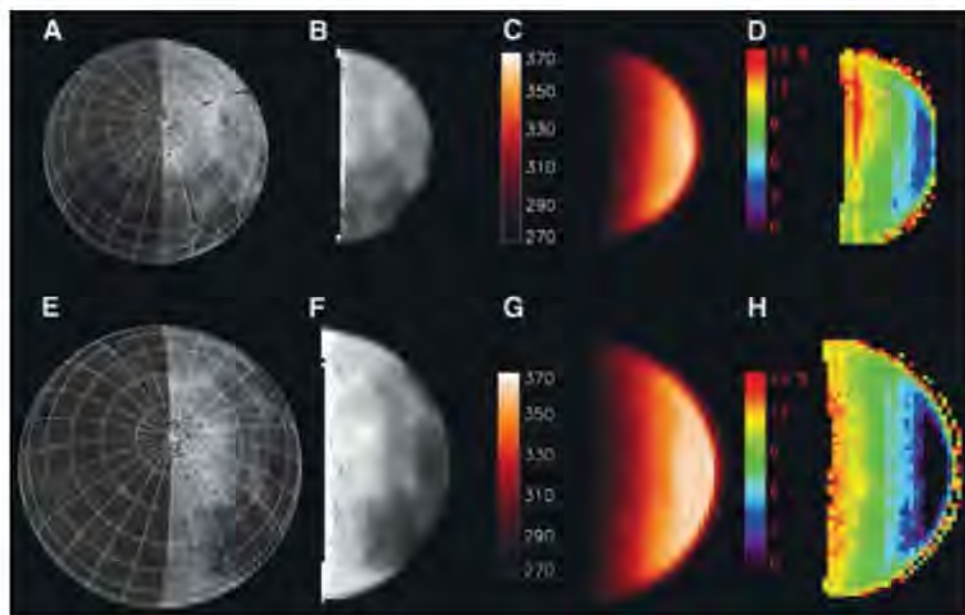
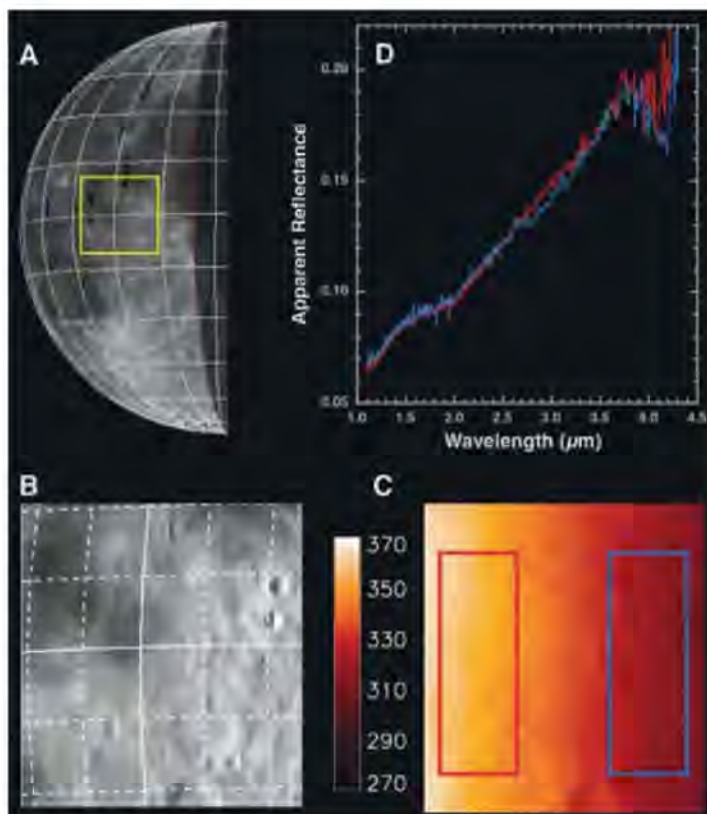


Fig. 2. Two sets of observations over the North Pole separated by one-quarter of a lunar day. Hydration is detected on both dates at all observable locations ($>10^\circ\text{N}$). (A to D) Data acquired on 2 June 2009 at $\sim 80\text{ km/pixel}$. (A) Clementine basemap (15° grid) of observed area. (B) The $1.2\text{-}\mu\text{m}$ Deep Impact albedo image. (C) Corresponding temperature map (in K) derived from $>4\text{-}\mu\text{m}$ spectra. (D) Corresponding map of the strength of the continuum-removed $2.8\text{-}\mu\text{m}$ hydration feature. (E to H) Data acquired on 9 June 2009 at $\sim 60\text{ km/pixel}$; images as in (A) to (D).

Fig. 3. Equatorial observations acquired on 27 December 2007 at 10 km/pixel . (A) Clementine basemap (15° grid) of observed area (yellow box). (B) The $1.2\text{-}\mu\text{m}$ Deep Impact albedo image (5° grid). Solid lines are the equator and prime meridian, which intersect in Sinus Medii. (C) Corresponding temperature map (in K) derived from $>4\text{-}\mu\text{m}$ spectra. (D) The average spectrum for most of the image lacks a hydration feature (e.g., red). However, regions nearer the evening terminator have weak, but distinct, $3\text{-}\mu\text{m}$ absorptions (blue). The red spectrum is scaled to match the reflectance of the blue spectrum. See boxes in (C) for locations.



tude, is the dominant factor determining OH and H₂O abundance.

Due to the rotation of the Moon, the morning terminator contained basaltic maria on 2 June and anorthositic highlands on 9 June. On both days, spectra along the terminator exhibit strong 3- μ m absorptions at all imaged latitudes, from 10°N to the North Pole, with the strongest absorptions at the pole (Fig. 2, D and H). The 2.8- μ m OH band depth values range from 9 to 11% for maria and 10 to 12% for highlands. These ranges, as well as comparisons of continuum-removed spectra of mare (Fig. 4A) and highland (Fig. 4B) units at similar latitudes along the terminator, show variations of only 1 to 2% band depth. We therefore observe no statistically significant compositional differences in maximum OH or H₂O content at our resolution of tens of kilometers.

The two June 2009 imaging scans also allow us to compare the same locations on the Moon at different local times and thus under different insolation conditions. For example, eastern Mare

Imbrium is at the morning terminator (relatively cold) on 2 June and near local noon on 9 June (Fig. 4E). Although the same area cannot be tracked from morning through evening, comparison of similar terrain types (highlands or maria) (Fig. 4, C to E) shows that all surfaces begin the day with comparable band depths (~11%), lose water as they move off the morning terminator, have a minimum absorption near noon (Fig. 4, C and D, black), and return to their morning values by evening. However, the highlands consistently retain more water (~50% of the morning band depth value) at their minimum near noon than do the maria (~30% of the morning band depth value). These differences in rates of hydration loss between maria and highlands presumably reflect differences in their mineralogy or grain size. Nonetheless, their common steady-state band depth indicates that there is no inherent difference in total OH or H₂O adsorptivity with composition. As seen in the continuum-removed spectra (Figs. 1 and 4), the overall shape of the

hydration feature also changed with decreasing strength; absorptions beyond 3 μ m decreased more than the 2.8- μ m OH feature. This nonlinear change with wavelength argues against geometric or photometric effects. Instead, it may represent wide variability in OH bond energies due to different cations. More likely, the change in the shape indicates that the absorptions beyond 3 μ m are attributable to H₂O, which is lost more readily than the relatively strongly bonded OH.

The strongest 3- μ m absorptions in the Deep Impact data occurred at the North Pole, where the 2.8- μ m OH band depth was 14% and the 3- μ m equivalent width was 0.07 μ m. Such high band depth values are surprising for an object as dark as the Moon because previous studies have shown that dark absorbing materials can cause a nonlinear decrease in the strength of hydration features (15–17). Using radiative transfer theory to account for these multiple scattering effects (18) and trends derived from laboratory data of synthetic OH- and H₂O-bearing basaltic glasses

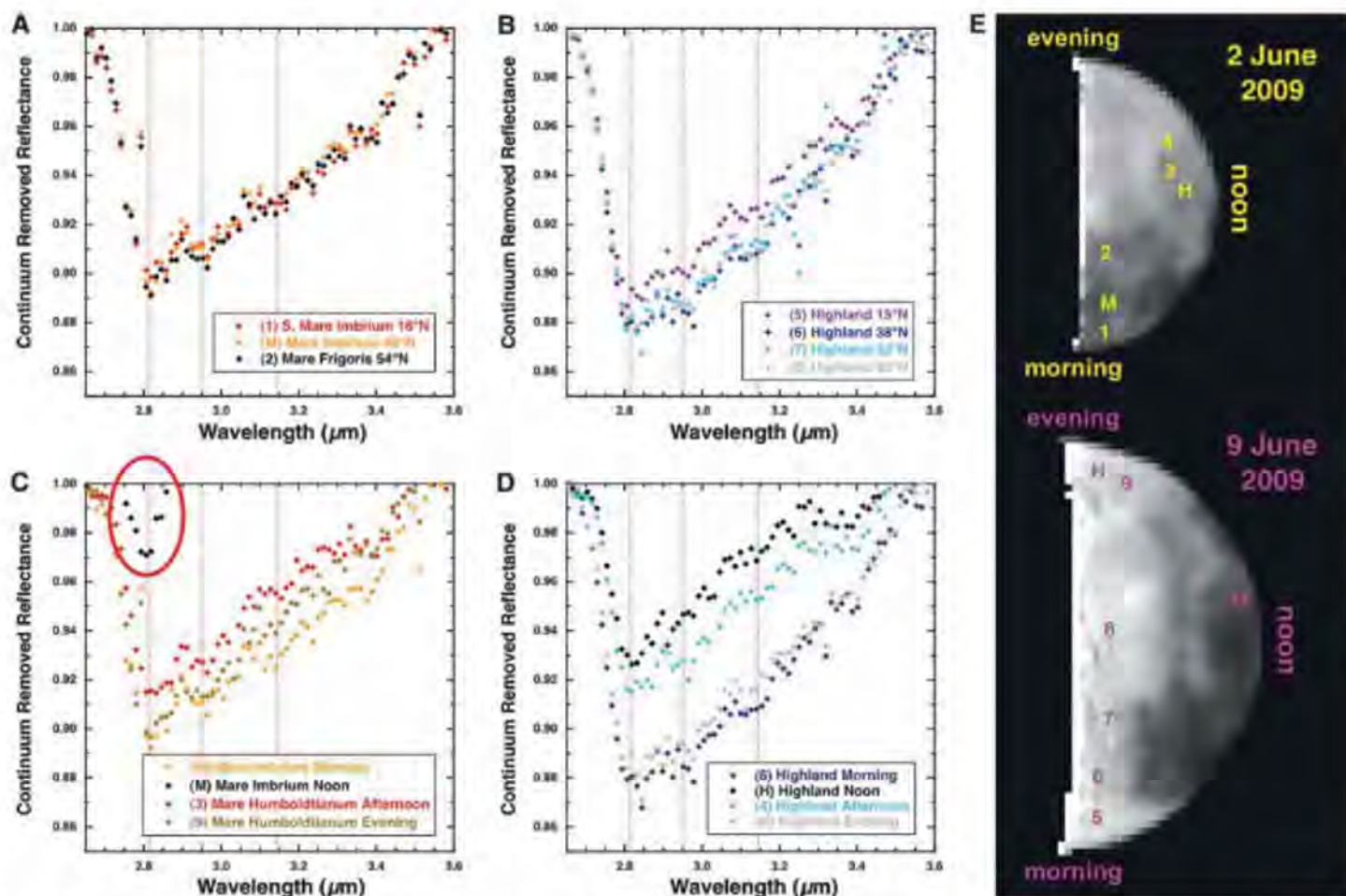


Fig. 4. Comparisons of mare and highland terrains as a function of latitude and time of day as observed over a quarter of a lunar day (90° rotation) from 2 and 9 June 2009. (A) Maria ranging from 16°N to 54°N along the morning terminator on 2 June have the same continuum-removed band depth and overall shape. Locations as indicated on (E). (B) Same as (A) for highland units ranging from 15°N to 80°N on 9 June. (C) The same location in eastern Mare Imbrium ("M") that is observed in the morning (orange) on 2 June rotates to noon (black; red circle) on 9 June with a 70% decrease in continuum-removed

band depth and a distinct change in shape. (D) The highland unit south of Mare Humboldtianum ("H") was observed at noon (black) on 2 June and in the evening (gray) on 9 June with only a 50% change in band depth. By evening, both mare (brown) and highland (gray) terrains return to their morning abundances (orange and blue, respectively). (E) Locations for terrains whose spectra are plotted on (A) to (D), on 2 June (top) and 9 June (bottom). The mare and highland units marked with "M" and "H" are visible in both data sets. All spectra are averages of 3 by 3 pixel areas.

(9), we estimate the water content at ~0.3 weight percent (wt %) (5). Similar values are obtained with trends derived from laboratory data of other hydrated phases [e.g., clay minerals, zeolites (9)], suggesting that the maximum volatile content of the bulk lunar soil is <0.5 wt %, regardless of the actual composition of the hydrated phase(s).

The Deep Impact observations of the Moon not only unequivocally confirm the presence of OH or H₂O on the lunar surface, but also reveal that the entire lunar surface is hydrated during at least some portions of the lunar day. Furthermore, the temporal variations observed over the lunar diurnal cycle reveal a dynamic hydration process driven by solar radiation. Our results suggest that it is likely that all areas of the Moon, regardless of composition or location, exhibit similar maximum hydration during the night. The short time scale of loss and recovery of OH or H₂O, a cycle that completes entirely within daylight hours, and the relatively low absolute abundances suggest that lunar hydration is a surface phenomenon. The rapid photodissociation of H₂O and the return to steady-state abundances during the day require a ready source, which would be consistent with a solar wind origin, as supported by the recent discovery of an extended source of water-group ions in the inner heliosphere (19). Over time, this daily hydration and dehydration

process may lead to a migration of OH and H⁺ toward the poles and their accumulation in permanently shadowed regions of the Moon (20). Such hydration via solar wind is expected to occur throughout the inner solar system on all airless bodies with oxygen-bearing minerals on their surfaces.

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Supporting Online Material

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Materials and Methods

Table S1

References

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Character and Spatial Distribution of OH/H₂O on the Surface of the Moon Seen by M³ on Chandrayaan-1

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The search for water on the surface of the anhydrous Moon had remained an unfulfilled quest for 40 years. However, the Moon Mineralogy Mapper (M³) on Chandrayaan-1 has recently detected absorption features near 2.8 to 3.0 micrometers on the surface of the Moon. For silicate bodies, such features are typically attributed to hydroxyl- and/or water-bearing materials. On the Moon, the feature is seen as a widely distributed absorption that appears strongest at cooler high latitudes and at several fresh feldspathic craters. The general lack of correlation of this feature in sunlit M³ data with neutron spectrometer hydrogen abundance data suggests that the formation and retention of hydroxyl and water are ongoing surficial processes. Hydroxyl/water production processes may feed polar cold traps and make the lunar regolith a candidate source of volatiles for human exploration.

The Moon had been believed to be quite dry since the return of lunar samples from the Apollo and Luna programs. Many Apollo samples contain some trace water or minor hydrous minerals, but these have typically been attributed to terrestrial contamination (see supporting online material text). A possible accumulation of volatiles, including water frost and ice, in the permanently shadowed regions of the lunar

poles has nevertheless been discussed for decades (1–3). The Lunar Prospector neutron spectrometer (LP-NS) directly measured H over the Moon and found a higher abundance associated with the permanently shadowed regions of both poles (4, 5), implying that the lunar poles could be potential cold traps for volatiles (6), some of which could be linked to solar-wind hydrogen (7). Here we present measurements acquired by the Moon

Mineralogy Mapper (M³) (8), a NASA instrument on Chandrayaan-1, India's first mission to the Moon, that show small amounts of OH/H₂O on the uppermost surface of the Moon.

The M³ spectrometer measures visible and near-infrared wavelengths, which contain highly diagnostic absorptions due to minerals as well as OH and H₂O (9) (fig. S1). Absorptions occur as solar radiation passes through multiple randomly oriented particles in the upper 1 to 2 mm of soil; reflectance spectra exhibit these combined absorptions from all particles. As soils evolve in the lunar environment, individual grains develop silicate glass coatings that contain nanophase metallic iron (npFe⁰) (10–12). The cumulative abundance of this weathering-derived npFe⁰ substantially de-

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creases the measured strength of all absorption bands of lunar material, especially for soils from the FeO-rich maria (13).

We have evaluated the 3- μ m spectral region in current M^3 data over the sunlit portion of the Moon to search for evidence of water. We detected a feature near the 3- μ m region in several areas of the first-returned sequences of M^3 data. As global data accumulated (Fig. 1), it became evident that this feature is observed systematically across the Moon. For various illumination geometries, the strength of the absorption feature near 3 μ m (Fig. 1B) is computed as a relative band depth = $1 - (Rb/Rc)$, where Rb is the average of channels at 2896 and 2936 nm, and Rc is the approximate continuum given by the average of channels at 2617, 2657, and 2697 nm. A small component of emitted thermal radiation often occurs along with reflected solar radiation in M^3 radiance measurements. When the surface is warm (greater than ~250 to 300 K), this added component is evident at wavelengths longer than 2000 nm. An iterative procedure to measure and remove this thermal emission component has been developed for M^3 data (14, 15). Surface temperature derived from the M^3 measurements are illustrated in Fig. 1C. As these M^3 measurements progressed across the lunar surface, the solar-illumination angle gradually decreased from east to west. For higher-temperature equatorial mare regions, the added thermal radiation could be up to 30% of the signal received at 3 μ m. Minor thermal emission may hide the presence of a weak absorption feature near 3 μ m, and although the relative band-depth image (Fig. 1B) excludes areas with detectable thermal radiation, it is a conservative limit to the distribution of the feature (8).

Although it is impossible to capture the full range of lunar surface mineralogy with three parameters, specific mineral properties displayed in red, green, and blue in Fig. 1D are directly linked to our understanding of the diagnostic absorptions of lunar materials (9, 16). Most importantly, these mineral patterns (that rely on spectral channels from the visible through ~2600 nm) do not appear to be correlated with M^3 measurements at slightly longer wavelengths (Fig. 1, B and C).

At the spatial resolution of these initial M^3 data (140 m per pixel), the 3- μ m feature is identified for soils at moderate to high latitudes (Fig. 1B), as well as at several fresh (plagioclase-rich) impact craters. In the highlands, small (<1 km) morphologically fresh impact craters have ejecta patterns with a prominent 3- μ m feature relative to their surroundings (fig. S2). More than one specific absorption may account for the spectral feature, and/or the distribution of the absorption appears variable with local conditions (effects of temperature, solar illumination). For example, Ryder crater, a fresh ~17-km Copernican-aged crater on the lunar farside (Fig. 2), and its immediate ejecta exhibit no discernable thermal emission component in M^3 data. Although most of the

crater exposes plagioclase-rich rocks, it is heterogeneous and contains regions with small amounts of Fe-bearing minerals (Fig. 2E). An enhanced 3- μ m band is seen in distal Ryder ejecta to the northwest. A diffuse and approximately inverse correlation of 3- μ m band strength with measured signal brightness is seen across the scene and suggests sensitivity to either solar-wind ions or solar insolation (i.e., small local variations of emissivity or other thermal effects). The traverse around the sunlit rim of Ryder crater illustrates this observation (see also fig. S3).

Individual M^3 spectra focusing on the 2000- to 3000-nm part of the spectral range acquired from highland terrain just east of the central meridian of the lunar nearside suggest that the relative strength of the 3- μ m feature increases with latitude (Fig. 3A). These spectra are derived from a current radiance calibration (version K) and modest image-based band-to-band calibrations with no thermal emission correction applied. Example spectra of several forms of H_2O and OH that might be seen with remote detectors are shown for comparison (Fig. 3B). The pattern seen in M^3 data may indicate increasingly strong OH/ H_2O absorptions with latitude, though we cannot eliminate the possibility that a 3- μ m feature is present at lower latitudes but is masked by a minor thermal emission component beyond 2.6 μ m (8).

We implemented several tests to validate the M^3 results for the 3- μ m region. We first scrutinized preflight laboratory calibrations of M^3 obtained at lunar operating temperatures and in a vacuum [see (17)]. Initial atmospheric effects were readily identified as the instrument reached vacuum equilibrium. Calibration data for two independent standards [spectralon, infragold (18)] were acquired. When applied to M^3 in-flight data, they produce results that do not alter the presence of the observed 3- μ m feature, but do alter its shape. We use the infragold standard because the spectralon has a weak feature near 2.8 μ m. Furthermore, using purely in-flight image-based methods that are independent of terrestrial laboratory calibration approaches, a feature near 3 μ m is seen in contrasting relative reflectance spectra.

At moderately high latitudes, there is sufficient orbit-to-orbit overlap that M^3 made repeat measurements of the same area on the lunar surface 2 hours apart under approximately the same illumination. The characteristics of observed spectral features were identical within data precision. Every month Chandrayaan-I passes the same location on the surface, but with a ~30° change in solar-illumination angle at the equator. We identified and processed four pairs of image strips across the western portion of the Orientale basin on the farside western limb that have nearly identical spatial coverage but substantially different illumination conditions. A local morning set was acquired in February 2009, and the second set was acquired two months later in the local afternoon. For several weeks between these optical periods, M^3 activated a decontamination heater to drive off any

condensed volatiles. Typical results are shown in Fig. 4, which depicts a region (~53°S, 259°E) that is low enough in temperature to exhibit no discernable thermal emission for sunlit areas in either geometry. Because of the large variations in local brightness, the spectra are scaled relative to an area exhibiting the weakest 3- μ m absorption. The presence of the 3- μ m feature was repeatable, but the time of lunar day does affect the apparent distribution and strength of the absorption.

Lastly, two independent spacecraft with spectrometers that extend beyond 3- μ m—Cassini in 1999 and Deep Impact in June 2009—have flown by the Moon for calibration purposes, and recent analysis of these data confirmed the presence of absorptions in spectra of the lunar surface similar to those reported here (19, 20). Because these spectrometers extend farther into the near-infrared than M^3 , they are able to more fully characterize the shape of the 3- μ m absorption, and this is important information to constrain the nature of the absorbing species.

Approximate abundance estimates of OH or H_2O are only possible with specific model assumptions about the physical form and location of the hydrated species. As one example, simple attenuation according to Beer's law might be assumed for a case where the detected water/ion is a surficial deposit on individual regolith grains, with the observed reflectance of the regolith dominated by sunlight absorbed and scattered by the grains themselves. Modeled abundance could be as high as 770 parts per million (ppm) (21) but is dependent on particle size, and the total abundance of hydrated material in the bulk upper regolith would be substantially smaller if hydration is not retained during regolith gardening.

The 3- μ m feature measured by M^3 originates from the upper few millimeters of the surface, whereas the bulk H detected by the LP-NS (22–24) represents the upper ~50 cm of the regolith. Because the spatial resolution of the LP-NS is two to three orders of magnitude lower than that of M^3 , only broad features on the order of hundreds of kilometers can be compared directly outside the permanently shadowed regions. Two distinct differences are noted: (i) In contrast to that seen for M^3 , the generally diffuse nature of LP-NS hydrogen for most regions on the Moon shows no pattern of widespread H present at high latitudes near the poles (<80°). (ii) Furthermore, some of the lowest regional abundance of LP-NS hydrogen corresponds to large expanses of anorthositic or freshly disturbed (immature) highland material (22, 23). One such example covered by both M^3 and LP-NS is the 113-km diameter crater Goldschmidt (73.0°N, 3.8°W) and its smaller fresh companion, the 51-km diameter Anaxagoras (73.4°N, 10.1°W). As in M^3 observations of many smaller feldspathic craters, M^3 data for Goldschmidt exhibit a prominent 3- μ m absorption (Fig. 1B), whereas in LP-NS data the region exhibits a distinctly low H abundance [see fig. S4 and (24)], suggesting that the hydrated materials observed by M^3 do not occur at depth.

Fig. 1. M^3 low-resolution-mode data for the lunar nearside acquired from Chandrayaan-1 in a 100-km orbit. All data have been spatially averaged by a factor of 100 and projected to produce a hemispheric overview (25). During this period of observations, the illumination geometry at the equator ranges from 45° in the east to 58° on the western limb. (A) Reflected light at 750 nm. Surface reflectance has not been adjusted for solar illumination, and brightness decreases toward the poles. (B) Measured $3\text{-}\mu\text{m}$ absorption strength. Brighter areas indicate strong absorption. The large northern crater near 0° longitude with strong $3\text{-}\mu\text{m}$ absorption is Goldschmidt (indicated with arrow). (C) Derived surface temperature (240 to 360 K). (D) Color composite designed to illustrate major mineral absorptions (red, reflectance at 1580 nm; green, integrated band depth near 1000 nm; blue, integrated band depth near 2000 nm after thermal removal). In this composite, the feldspathic highlands appear largely red (with a few mafic minerals), whereas the basaltic maria are variations of green, light blue, and dark blue, illustrating the presence and diversity of mafic minerals.

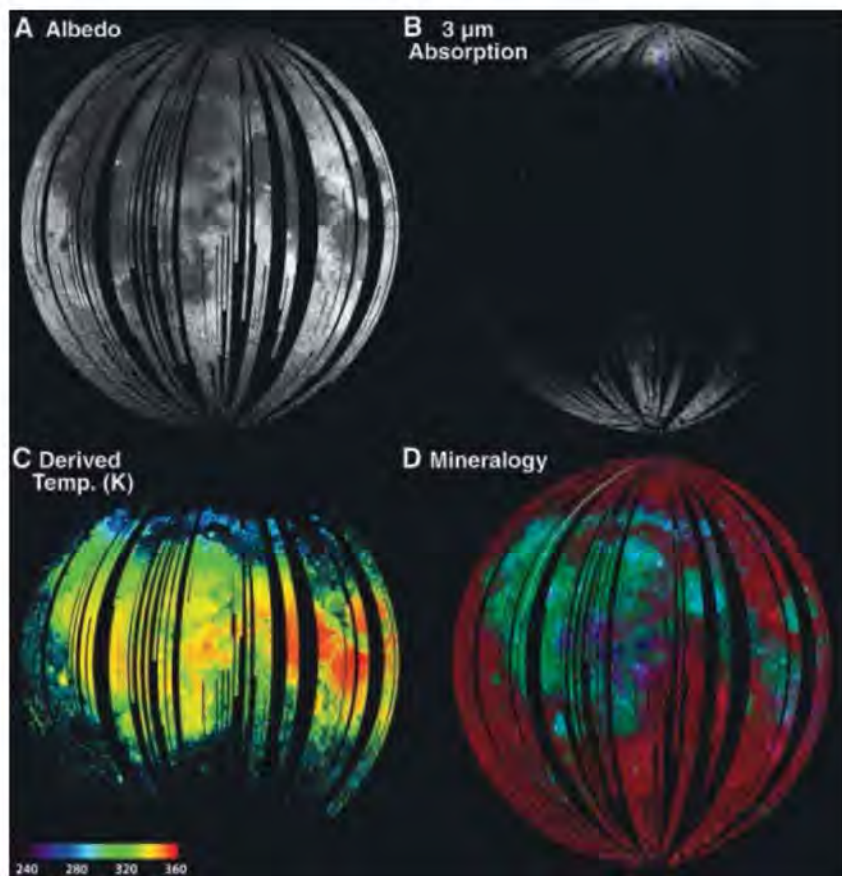


Fig. 2. Subscene of M^3 strip M3G20090125T172600 including Ryder crater (at right), centered at 143.2°E longitude and 44.5°S latitude. (A) Brightness image at 2856 nm. (B) Image of the relative $3\text{-}\mu\text{m}$ feature depth for the same area. A coherent spatial distribution is seen associated with Ryder ejecta, as well as a topography-related pattern of solar insolation. (C) Data cloud for the subscene comparing the relative $3\text{-}\mu\text{m}$ feature depth and near-infrared reflectance. Some regions show a diffuse inverse-correlation as a function of apparent reflectance (26). (D) Location of spectra collected along the wall of Ryder crater, from well-illuminated areas (1) to more shaded areas (7 and 8). (E) M^3 spectra for the areas shown in (D). Although Ryder crater exhibits spatially diverse lithologies, the strength of the $3\text{-}\mu\text{m}$ feature is weakest in full sun and increases as solar illumination decreases until the lithology changes (becomes more mafic), a pattern that might be indicative of variations of infilling from minor thermal emission (8).

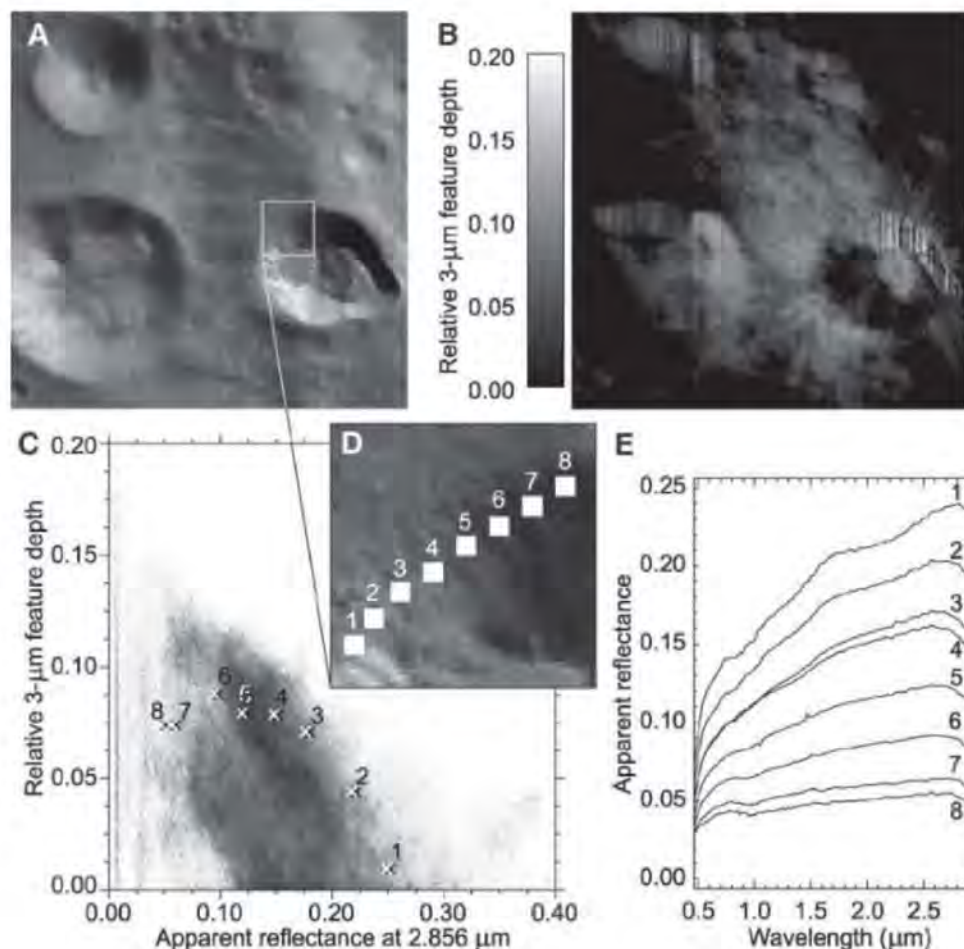
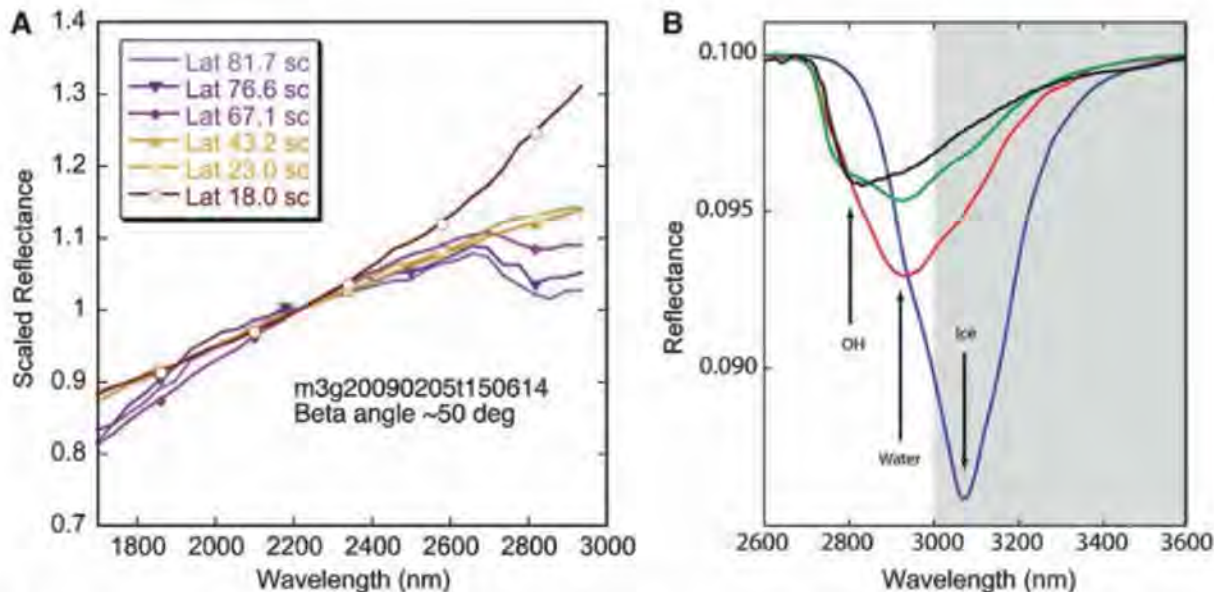
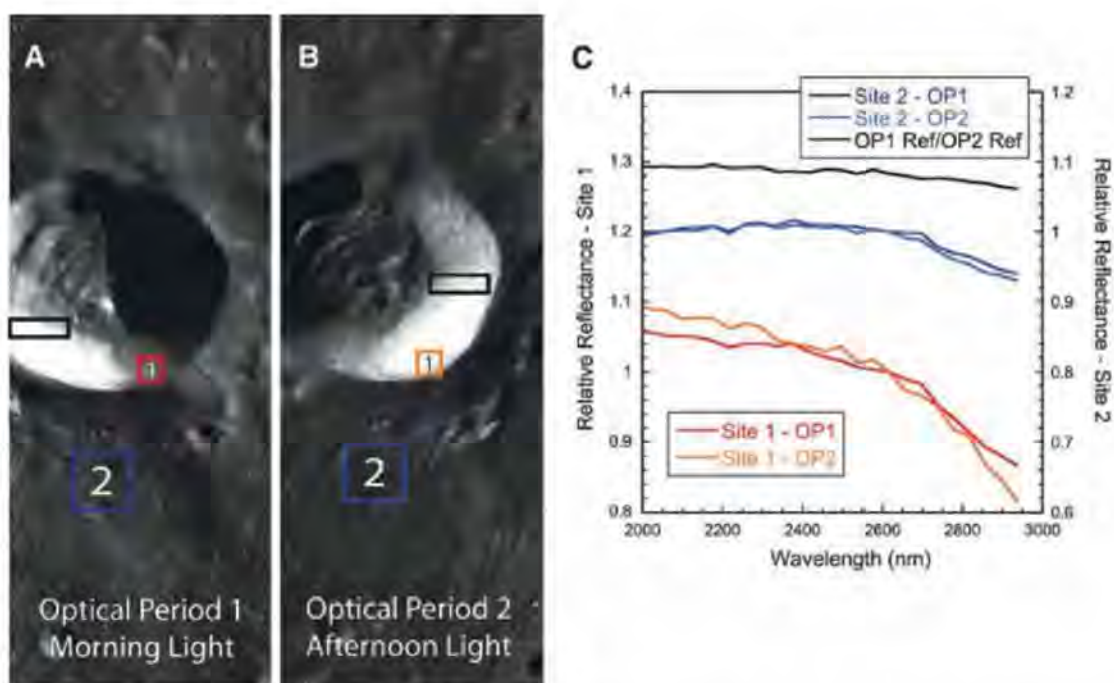


Fig. 3. (A) Scaled reflectance spectra for M³ image strip M3G200902005T-150614. All spectra are 7-by-7-pixel averages, and no thermal emission has been removed to allow the measured flux to be compared. The strongest detected 3- μ m feature (~10%) occurs at cool, high latitudes, and the measured strength gradually decreases to zero toward mid-latitudes (where thermal emission is necessarily less well constrained by M³). At lower latitudes (18°), the additional thermal emission component becomes evident at wavelengths above ~2200 nm (14, 15). **(B)** Model near-infrared reflectance spectra of H₂O and OH applicable for lunar comparisons. These spectra are highly dependent on physical state. A model of a thin layer of H₂O water (red) and ice (blue) on a 10% reflective surface equivalent to ~1000-ppm abundance is distinct from



anorthite (green) and a lunar glass analog (black). The shaded area extends beyond the spectral range of M³. Calculations are based on optical constants from (27–29), assuming no scattering in the H₂O or OH and with a 100- μ m path length within the substrate.

Fig. 4. M³ data taken two months apart during morning **(A)** [optical period 1 (OP1)] and afternoon **(B)** [optical period 2 (OP2)] solar illumination. The large 30-km Chadwick crater is located on the farside at 258.7°E, 52.7°S. Spectra in panel **(C)** are scaled reflectance for areas 1 and 2, relative to a local area of strong solar illumination that exhibits a relatively weak 3- μ m band in the scene (this reference location varies with geometry). Background soil region 2 (50 by 50 pixels) exhibits a moderately weak and consistent 3- μ m band strength. Region 1 within the crater (20 by 20 pixels) exhibits a more prominent apparent band strength, perhaps sensitive to solar illumination. Black boxes in **(A)** and **(B)** (50 by 15 pixels) indicate that the reference area selected for spectral ratios.



The M³ 3- μ m data clearly indicate that a minor hydrated phase or hydration process occurs on the lunar surface. This finding could imply that the Moon contains primary hydrated mineral phases that are uncommon in the limited Apollo, Luna, and lunar meteorite collections. These unsampled phases might be endogenic to the Moon and freshly exposed by craters in ancient highland terrain, or they may form during an impact event by a water-bearing comet or asteroid. On the other hand, H₂O and OH species might also be continuously created when solar-wind protons interact with the oxygen-rich surfaces during the formation of

lunar soil particles. In addition, fresh broken surfaces and soil grains may readily react with protons from the solar-wind, forming strong surficial OH bonds. Either of these may be highly dependent on the temperature and solar-illumination environment. The differences described between LP-NS hydrogen abundance and M³ 3- μ m band depth (linked to OH/H₂O abundance) imply that the M³ detection of OH/H₂O species is distinctly surface-correlated (i.e., linked to the upper few millimeters of the lunar regolith), but not substantially deeper. Thus, surficial processes involving the solar-wind are the most likely explanation of our observations.

The process for producing OH/H₂O on the Moon may provide an ongoing mechanism for delivery of these volatile elements to cold traps in the polar permanently shadowed regions. Perhaps most importantly, harvesting the lunar regolith for volatiles now becomes a serious option for long-term human activities.

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16. Bright feldspathic (highland) soils appear relatively red in this image. The green and blue displayed channels (green–light blue–dark blue colors in Fig. 1D) capture the relative band strength of diagnostic absorptions of several ferrous minerals. Regions containing abundant and diverse mafic minerals are highlighted by these two parameters. Both are calculated relative to a continuum and are integrated across M^3 channels within the broad mineral absorption bands (see fig. S1). The strength of these particular parameters is largely sensitive to the relative abundance of pyroxene, but the integrated 1000-nm band-strength parameter (green) is also sensitive to other mafic minerals present (e.g., olivine). The band strengths of all absorptions are modulated by the abundance of opaque phases (ilmenite, chromite) and $npFe^0$.
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21. A measured depth of the 3- μ m absorption band of ~7% would be equivalent to an effective thickness of ~0.09 μ m for such surficial water, which could be accounted for by a single molecular layer (or a few layers) of water/ion on individual grains, given an effective particle size for the interacting upper lunar regolith of ~1 μ m and an overall optical depth of ~300 μ m. A monolayer of water/ion would not tend to migrate into the grains, which would therefore remain dry (i.e., the overall abundance of water in the surface would remain low but potentially stable). If this surficial water/ion were photolytically dissociated, the resulting H ions may simply migrate along the surface until interacting with another O atom in the grain to again form OH or H_2O , resulting in an effective pseudostability or temporary steady state. If the regolith grains are anorthositic plagioclase, a monolayer of water on a 1- μ m spherical grain would equate to a molar abundance of 0.6% and a ~770-ppm mass fraction, given a 1/1 ratio of water molecule/ion to the anorthositic surface molecules. However, if the effective particle size of regolith grains carrying the water is larger than 1 μ m, then the surface water proportion will be less.
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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S5

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Smoothened Mutation Confers Resistance to a Hedgehog Pathway Inhibitor in Medulloblastoma

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The Hedgehog (Hh) signaling pathway is inappropriately activated in certain human cancers, including medulloblastoma, an aggressive brain tumor. GDC-0449, a drug that inhibits Hh signaling by targeting the serpentine receptor Smoothed (SMO), has produced promising anti-tumor responses in early clinical studies of cancers driven by mutations in this pathway. To evaluate the mechanism of resistance in a medulloblastoma patient who had relapsed after an initial response to GDC-0449, we determined the mutational status of Hh signaling genes in the tumor after disease progression. We identified an amino acid substitution at a conserved aspartic acid residue of SMO that had no effect on Hh signaling but disrupted the ability of GDC-0449 to bind SMO and suppress this pathway. A mutation altering the same amino acid also arose in a GDC-0449-resistant mouse model of medulloblastoma. These findings show that acquired mutations in a serpentine receptor with features of a G protein–coupled receptor can serve as a mechanism of drug resistance in human cancer.

The Hh signaling pathway has been implicated in the pathogenesis of human basal cell carcinoma (BCC) and medulloblastoma (1, 2). Constitutive Hh signaling, which

is most often due to underlying loss-of-function mutations in the gene encoding the inhibitory receptor Patched 1 (*PTCH1*), occurs in a majority of BCCs and approximately 30% of sporadic

medulloblastoma cases (2, 3). Mice that are heterozygous for *Ptch1* (*Ptch1*^{+/−}) spontaneously develop medulloblastoma, and treatment with Hh pathway inhibitors suppresses tumor growth and prolongs survival (4, 5). The Hh pathway inhibitor GDC-0449, a 2-pyridyl amide initially identified in a high-throughput screen, targets the G protein–coupled-like receptor Smoothed (SMO), which becomes activated after loss of *PTCH1* function (3, 6–8). In early-stage clinical studies, administration of GDC-0449 to patients with advanced BCC has produced promising results, highlighted by a 55% overall response rate (6, 7). In addition, treatment of a medulloblastoma patient harboring widespread metastatic disease with GDC-0449 resulted in a rapid and dramatic tumor regression (8).

Molecular profiling of the medulloblastoma patient's primary and metastatic tumor taken before treatment with GDC-0449 revealed an underlying somatic mutation in *PTCH1* (*PTCH1*-W844C) as well as up-regulated expression of Hh pathway target genes, supporting the hypothesis that the tumor was driven by dysregulated

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Hh signaling (fig. S1) (8, 9). The *PTCH1*-W844C mutation was not capable of suppressing SMO activity in a Hh-responsive, *GLI*-luciferase reporter cell line (C3H10T $\frac{1}{2}$ fibroblasts) when cotransfected together with wild-type (WT) SMO, indicating that this specific mutation can inhibit the ability of *PTCH1* to repress SMO and thus lead to aberrant, ligand-independent activation of the Hh pathway (fig. S2) (10). Despite the marked tumor shrinkage initially observed in this patient, PET scans taken ~3 months after initiation of treatment indicated disease progression. A fine needle aspirate of a progressing lesion was obtained for confirmation of disease recurrence and for subsequent molecular profiling so as to explore mechanisms of acquired resistance to GDC-0449. Sequencing of *PTCH1* confirmed the presence of the previously detected homozygous *PTCH1*-W844C mutation, which was accompanied by loss of heterozygosity (fig. S1).

To characterize the mechanism of relapse, we evaluated the status of known components of the Hh pathway, including *SMO*, the direct target of GDC-0449. We did not detect amplification of the *SMO* locus in this specimen (fig. S3) but identified a heterozygous G-to-C missense muta-

tion at position 1697, which is predicted to change codon 473 from Asp to His (D473H) (Fig. 1A). This change was not detected in the primary disease specimen. Using mass spectrometry-based genotyping, we detected the mutant allele only in the biopsy taken after relapse but not in normal skin from this individual or in the primary and metastatic disease biopsies taken before treatment with GDC-0449 (fig. S4). By deep sequencing, the mutant allele was not detected at an allele frequency of $\geq 0.1\%$ in either the primary or metastatic disease biopsy obtained before treatment with GDC-0449 (10). The mutant allele was also not detected by mass spectrometry-based genotyping of 64 banked medulloblastoma specimens.

To study the functional consequences of this mutation, we cotransfected C3H10T $\frac{1}{2}$ cells with expression vectors encoding SMO-WT or SMO-D473H together with a Hh-responsive *GLI*-luciferase reporter construct. SMO-WT and SMO-D473H were expressed at similar levels as determined with Western blotting (fig. S5) and fluorescence-activated cell sorting (FACS) analysis (fig. S6). SMO-D473H transfection induced Hh pathway activity to levels comparable with that seen with

SMO-WT, demonstrating that SMO-D473H is fully capable of activating Hh signaling (Fig. 2A). However, in contrast to the constitutively active mutant SMO-M2 (11), the activity of SMO-D473H was not substantially higher than SMO-WT and demonstrated a similar sensitivity as SMO-WT to *PTCH1* inhibition, suggesting that SMO-D473H may not have inherent oncogenic potential and will only activate Hh signaling in the absence of *PTCH1*. To determine whether this mutation impedes the ability of GDC-0449 to inhibit Hh signaling, we measured the half maximal concentration (IC_{50}) of drug required to inhibit *GLI*-luciferase reporter activity (Fig. 2B). Although GDC-0449 inhibited reporter activity at an IC_{50} of 20 nM in SMO-WT-transfected cells, no inhibition was observed in SMO-D473H-transfected cells even at concentrations as high as 3 μ M; this finding suggests that the mutation confers resistance to GDC-0449 without affecting the ability of SMO to transmit the Hh signal. SMO-D473H also impaired the ability of a chemically divergent SMO inhibitor, KAAD-cyclopamine (12), to inhibit *GLI*-luciferase reporter activity with a 43-fold change in IC_{50} (fig. S7). Lastly, we addressed whether the D473H mutation affected the receptor's ability to bind GDC-0449. Whereas 14 C-labeled GDC-0449 specifically bound to SMO-WT, it showed no specific binding to SMO-D473H (Fig. 2C). Thus, the inability of GDC-0449 to suppress Hh signaling in the context of the SMO-D473H mutation is associated with a deficiency in drug binding.

To further explore potential mechanisms of GDC-0449 resistance in medulloblastoma in vivo, we developed drug-resistant, subcutaneous allograft derivatives of medulloblastoma tumors from *Ptch1* $^{+/-}$; *p53* $^{-/-}$ mice (5) through intermittent dosing until tumors no longer responded to twice-daily dosing of GDC-0449. Using this approach, we established three separate drug-resistant tumor lines, of which one model (SG274) is described here (Fig. 3A). Sequencing of *Smo* in the SG274 model revealed a heterozygous A-to-G

Fig. 1. Identification of a *SMO* mutation in tumor samples from a medulloblastoma patient who relapsed after an initial response to GDC-0449. (A) Nucleotide sequence tracings showing a heterozygous mutation in *SMO* causing a Asp>His change at amino acid 473 (asterisk). This mutation was present in a metastatic biopsy taken at relapse but was not present in the primary tumor before GDC-0449 treatment. (B) The GPCR architecture of SMO maps the location of the D473H mutation to the C-terminal end of TM6. Looking down at the extracellular face of the GPCR helix bundle (color-ramped from TM1 in blue to TM7 in red, with ectoloops left out for clarity), a molecular model of SMO built upon the rhodopsin [Protein Data Bank (PDB) number 2Z73] and β 1-adrenergic receptor template (PDB number 2VT4) with MODELLER (18) shows the position of the Asp-473 residue facing the central binding cavity.

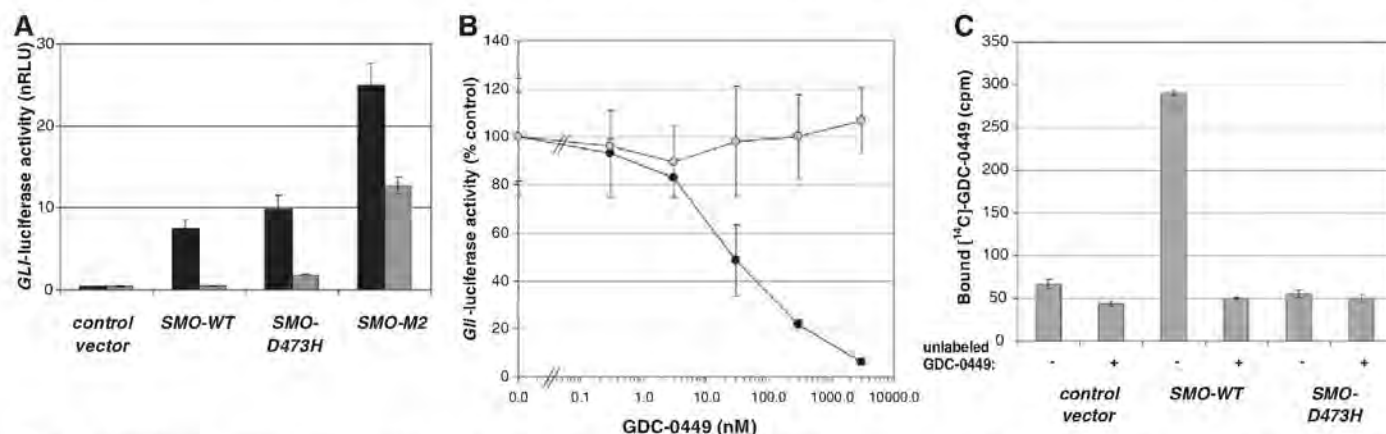
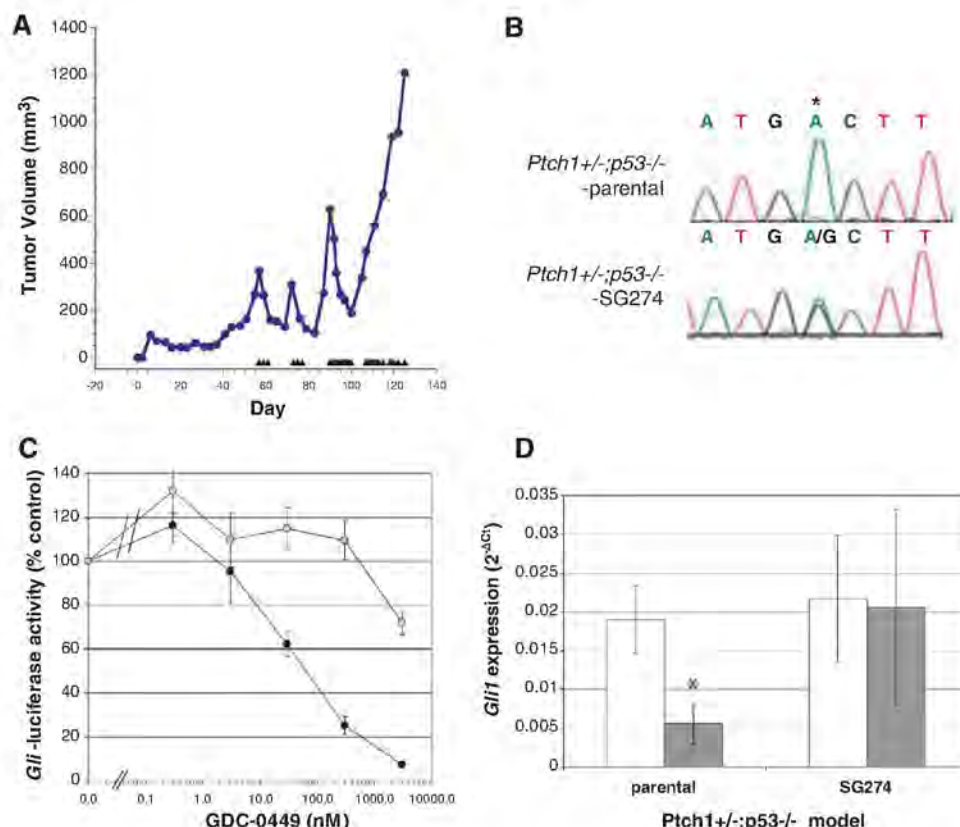


Fig. 2. The SMO-D473H mutation confers resistance to the Hh pathway inhibitor GDC-0449. (A) *GLI*-luciferase reporter activity after transfection of SMO variants in the presence (gray bars) or absence (black bars) of *PTCH1* DNA (20 ng). *SMO*-M2 represents a previously identified activating mutation. (B) *GLI*-luciferase reporter activity in C3H10T $\frac{1}{2}$ cells transfected with SMO-WT (closed circles) or

SMO-D473H (open circles) after treatment with various doses of GDC-0449. Reporter activity is normalized to untreated cultures. (C) Binding of 14 C-labeled GDC-0449 (5 nM) to human embryonic kidney-293 cells transfected with SMO variants in the presence or absence of unlabeled GDC-0449 (5 μ M), to demonstrate specificity. Data in all experiments represent mean $\pm$ SD.

Fig. 3. Acquired resistance to GDC-0449 through *Smo* mutation in a genetically engineered mouse model of medulloblastoma. **(A)** Medulloblastoma allografts from *Ptch1*^{+/+};*p53*^{-/-} mice were made GDC-0449-resistant through intermittent daily dosing with 75 mg/kg GDC-0449. Treatment days are represented by triangles, and tumors were excised once they failed to respond to twice-daily dosing with GDC-0449. **(B)** Nucleotide sequence tracings from parental and a GDC-0449-resistant (SG274) medulloblastoma allografts showing a heterozygous mutation resulting in a D>G change at amino acid 477 of *Smo* (homologous to position 473 of human *SMO*). **(C)** *Gli1*-luciferase reporter activity in C3H10T1/2 cells transfected with *SMO*-WT (closed circles) or *SMO*-D473G (open circles) after treatment with various doses of GDC-0449. **(D)** Quantitation of *Gli1* mRNA levels by quantitative reverse transcription polymerase chain reaction from multiple (*n* = 3) tumors collected 6 hours after treatment with vehicle control (open bars) or 75 mg/kg GDC-0449 (closed bars) from parental and SG274 tumor-bearing mice. Data indicate mean $\pm$ SD. **P* < 0.05 (*t* test).



missense mutation at position 1944, resulting in aspartic acid-477 to glycine (D477G) change, which was not identified in the parental GDC-0449-sensitive model (Fig. 3B). Strikingly, the corresponding residue in human *SMO* is the aspartic acid at position 473 that was mutated in the relapsed medulloblastoma patient (fig. S8). Approximately 100-fold more GDC-0449 is needed to suppress Hh signaling in cells that ectopically express the glycine variant at this position as compared with that in WT cells (Fig. 3C). Furthermore, GDC-0449 did not suppress Hh signaling in vivo, as demonstrated by the inability of GDC-0449 to down-regulate *Gli1* levels in SG274 tumors subcutaneously implanted in mice (Fig. 3D). Data from this mouse model thus provide additional evidence that mutation of *SMO* at this specific aspartic acid residue can confer resistance to GDC-0449. Additional mechanisms of resistance to GDC-0449 exist because *Smo* mutations were not identified in the other two models.

Topology prediction and structural modeling of *SMO* map the Asp-473 residue to the C-terminal end of the sixth transmembrane segment (TM6), a position that is highly conserved across *SMO* orthologs and the related Frizzled family of Wnt receptors (Fig. 1B and fig. S8). The heptahelical structure of *SMO* is required for binding of cyclopamine (13) and is the target for ortho- and allosteric G protein-coupled receptor (GPCR) modulators (14). Because Asp-473 is positioned at the extracellular lip of the central cavity formed by the canonical GPCR architecture (15) of *SMO*, the nonconservative

mutation of this residue may potentially destabilize the packing of *SMO* ectoloops or the inner topography of the protected binding pocket.

In summary, our study provides a proof of principle that GPCR-like proteins can become drug resistant through the acquisition of genetic mutations. These findings have direct implications for the clinical development of *SMO* inhibitors in tumors in which the Hh pathway is mutated and may be applicable to future GPCR targets in cancer because many have been shown to play a critical role in tumor growth and metastasis (16). Furthermore, the demonstration that these mutations do not impact Hh signaling continues to support the rationale for targeting this pathway but also highlights the need to either identify second-generation *SMO* inhibitors capable of overcoming acquired resistance, identify inhibitors targeting downstream signaling molecules (17), or potentially initiate earlier treatment before therapy with radiation or other DNA-damaging agents.

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Figs. S1 to S8
References

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A Cdc20-APC Ubiquitin Signaling Pathway Regulates Presynaptic Differentiation

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Presynaptic axonal differentiation is essential for synapse formation and the establishment of neuronal circuits. However, the mechanisms that coordinate presynaptic development in the brain are largely unknown. We found that the major mitotic E3 ubiquitin ligase Cdc20-anaphase promoting complex (Cdc20-APC) regulates presynaptic differentiation in primary postmitotic mammalian neurons and in the rat cerebellar cortex. Cdc20-APC triggered the degradation of the transcription factor NeuroD2 and thereby promoted presynaptic differentiation. The NeuroD2 target gene encoding Complexin II, which acts locally at presynaptic sites, mediated the ability of NeuroD2 to suppress presynaptic differentiation. Thus, our findings define a Cdc20-APC ubiquitin signaling pathway that governs presynaptic development, which holds important implications for neuronal connectivity and plasticity in the brain.

The establishment of neuronal circuitry during brain development requires the formation of synapses between neurons, and an

essential part of this is presynaptic axon differentiation. Synapse development is regulated by the ubiquitin-proteasome pathway (1, 2). How-

ever, an E3 ubiquitin ligase that orchestrates presynaptic differentiation in the mammalian brain remained to be identified.

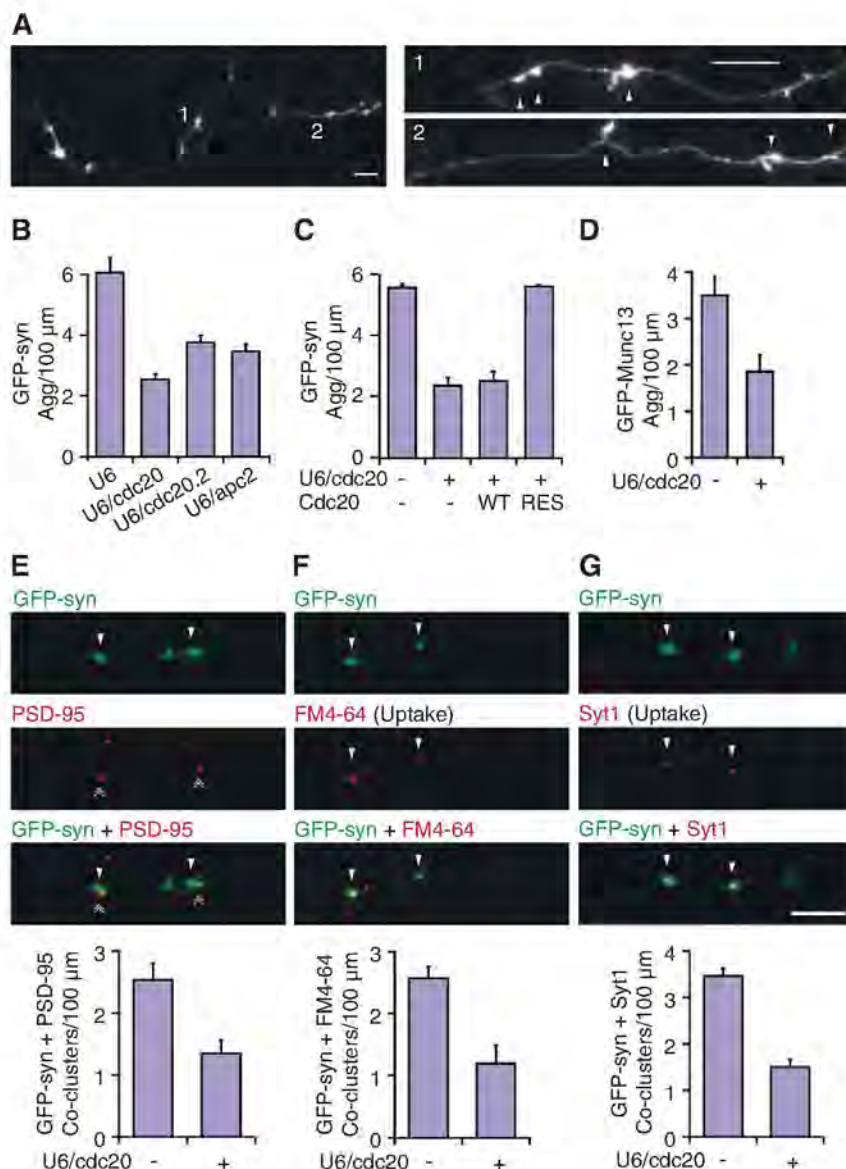
The cell cycle-regulated ubiquitin ligase, the anaphase-promoting complex (APC), is highly expressed in postmitotic mammalian neurons (fig. S1A) (3, 4). The APC associates with the critical coactivator Cdc20 or Cdh1 (3, 4). Although Cdh1-APC controls axon growth and patterning (3), it does not appear to regulate the number of presynaptic sites in rat cerebellar granule neurons (fig. S13). We thus asked whether Cdc20-APC plays a role in presynaptic differentiation.

To study presynaptic development, we first characterized clustering of the synaptic vesicle protein

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Fig. 1. Cdc20-APC induces presynaptic differentiation in postmitotic neurons. **(A)** Image of a cerebellar granule neuron transfected with a GFP-synapsin expression plasmid. Distal axons are shown in right panels. Arrowheads denote synapsin clusters. **(B)** Knockdown of Cdc20 (U6/cdc20 or U6/cdc20.2) or APC2 (U6/apc2) decreased synapsin cluster density in granule neurons ($P < 0.01$, analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) post hoc test, $n = 3$). **(C)** Expression of Cdc20-RES, but not Cdc20-WT, increased synapsin clustering in the background of Cdc20 RNAi ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). **(D)** Cdc20 knockdown decreased Munc13 clusters ($P < 0.01$, t test, $n = 3$). **(E)** (Top) granule neurons expressing GFP-synapsin were subjected to immunocytochemistry using GFP and PSD-95 antibodies. Arrowheads indicate representative synapsin/PSD-95 coclusters. (Bottom) Cdc20 knockdown reduced the density of synapsin/PSD-95 coclusters ($P < 0.05$, t test, $n = 3$). **(F and G)** (Top) Granule neurons expressing GFP-synapsin were analyzed for synaptic vesicle recycling using the uptake of the dye FM4-64 or a luminal Syt1 antibody. Arrowheads indicate representative synapsin and FM4-64 or Syt1 coclusters. (Bottom) Cdc20 knockdown reduced the density of synapsin coclustered with FM4-64 ($P < 0.05$, t test, $n = 3$) or Syt1 ($P < 0.001$, t test, $n = 3$) uptake sites. Scale bars, 20 μ m.



synapsin in primary granule neurons isolated from the rat cerebellar cortex. To visualize synapsin clustering, we transfected granule neurons with an expression plasmid encoding synapsin fused to green fluorescent protein (GFP-synapsin) (5). We observed GFP-synapsin clusters preferentially in the distal portion of axons, and these clusters overlapped with the endogenous synaptic vesicle proteins VAMP2 and VGlut1 (Fig. 1A and fig. S1, B to D).

To determine the role of Cdc20-APC in synapsin clustering, we induced knockdown of Cdc20 in neurons using two independent short hairpin RNAs (shRNAs) (fig. S2A) (4). Synapsin clustering was robustly reduced in Cdc20 knockdown neurons (Fig. 1B and fig. S2B). Expression of a rescue form of Cdc20 encoded by an RNA interference (RNAi)-resistant cDNA (Cdc20-RES), but not Cdc20 encoded by wild-type cDNA (Cdc20-WT), reversed the Cdc20 RNAi-induced loss of synapsin clusters in neurons, suggesting that the Cdc20 RNAi-induced phenotype is the result of specific knockdown of Cdc20 in neurons (Fig. 1C and fig. S2C) (4). Cdc20 knockdown in cerebellar slices also reduced synapsin cluster density in granule neuron parallel fiber axons (fig. S3). In

other experiments, knockdown of the core APC subunit APC2 in granule neurons reduced the density of synapsin clusters (Fig. 1B and fig. S2, A and B). Together, these results suggest that Cdc20-APC drives synapsin clustering in postmitotic neurons.

We asked whether Cdc20-APC regulates other aspects of presynaptic differentiation. GFP-synapsin clusters colocalized with clusters of endogenous active zone proteins, including Munc13, Bassoon, Rim1, and Liprin- α (fig. S4A). Accordingly, knockdown of Cdc20 triggered the loss of GFP-Munc13 clusters along the axon in granule neurons (Fig. 1D and fig. S4B). Thus, Cdc20-APC promotes the coordinate clustering of synaptic vesicle and active zone proteins.

We next assessed whether Cdc20-APC promotes the differentiation of functional presynaptic sites that form synapses. Synapsin clusters were apposed to clusters of the postsynaptic protein PSD-95, and Cdc20 knockdown reduced the density of synapsin/PSD-95 coclusters in granule neurons (Fig. 1E). In other experiments in which we measured the ability of axons to undergo synaptic vesicle recycling, synapsin clusters were found to be colocalized with sites of uptake of the dye FM4-64 or a luminal Syt1 antibody (6, 7), and Cdc20

knockdown reduced the density of synapsin coclusters with FM4-64 or Syt1 uptake sites (Fig. 1, F and G). Collectively, our results suggest that Cdc20-APC drives the differentiation of presynaptic sites that are functionally active and that form synapses.

Because the ubiquitin ligase activity of Cdc20-APC is critical for its function, we reasoned that neuronal Cdc20-APC promotes presynaptic differentiation by inducing the degradation of a protein that inhibits presynaptic development. The brain-enriched transcription factor NeuroD2 harbors the Cdc20 recognition motif, the destruction box (D-box) (8), suggesting that NeuroD2 might be a target of Cdc20-APC in the control of presynaptic development. NeuroD2 protein levels decreased in the developing rat cerebellum and in primary granule neurons with maturation, inversely correlating with the increasing levels of Cdc20 (Fig. 2A and fig. S5, A and B). Endogenous NeuroD2 levels increased in granule neurons treated with the proteasome inhibitor MG132 (Fig. 2B). In other experiments, NeuroD2 was found to be conjugated with ubiquitin in neurons (Fig. 2C). Together, these results suggest that NeuroD2 is regulated by the ubiquitin-proteasome system.

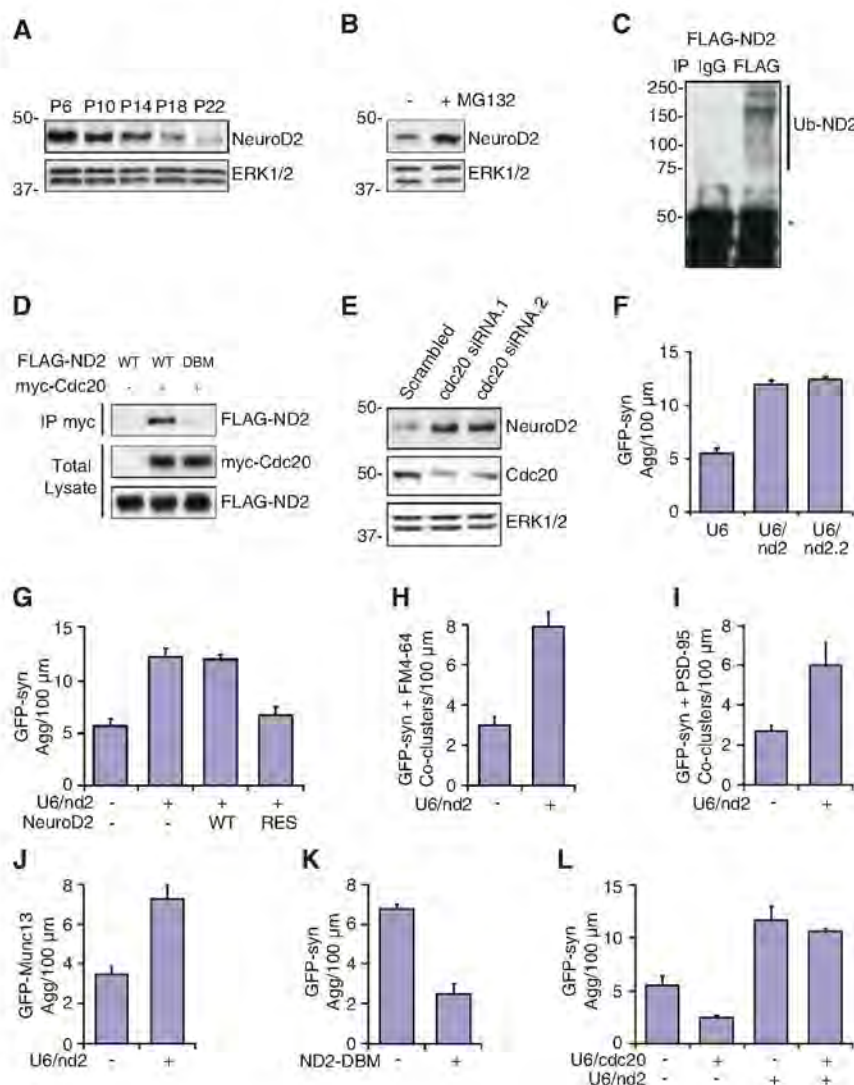
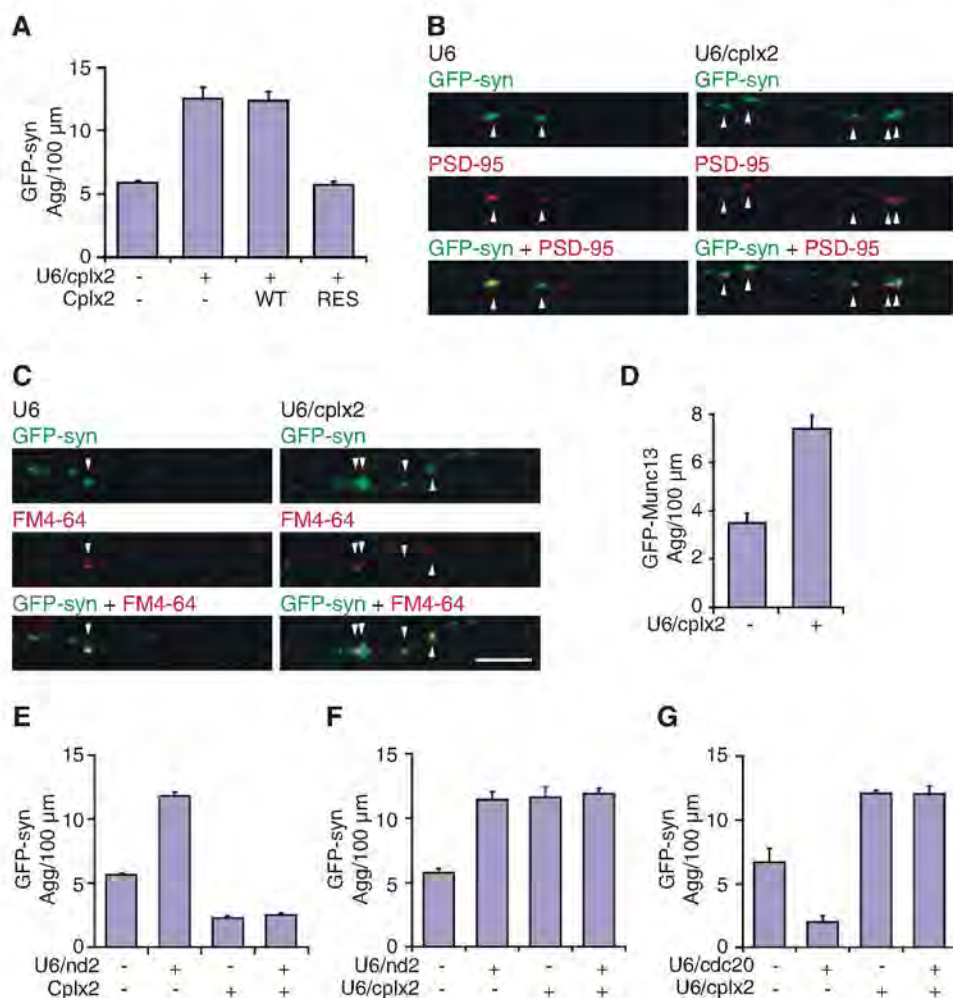


Fig. 2. Cdc20-APC-induced degradation of NeuroD2 drives presynaptic differentiation. (A) Lysates of cerebella from rat pups at postnatal days 6, 10, 14, 18, and 22 were immunoblotted with antibody to NeuroD2 or extracellular signal-regulated kinase 1/2 (ERK1/2). (B) Lysates of granule neurons treated with MG132 (5 μ M) or vehicle were immunoblotted with the NeuroD2 or ERK1/2 antibody. (C) Lysates of granule neurons transfected with the FLAG-NeuroD2 expression plasmid were immunoprecipitated with the FLAG antibody or control immunoglobulin G followed by immunoblotting with the ubiquitin antibody. (D) Lysates of 293T cells transfected with the FLAG-ND2-WT or FLAG-ND2-DBM expression plasmid together with the myc-Cdc20 expression plasmid or its control vector were immunoprecipitated with the myc antibody followed by immunoblotting with the FLAG antibody. Total lysates were immunoblotted with the myc or FLAG antibody. (E) Lysates of granule neurons transfected with two different small interfering RNAs (siRNAs) targeting Cdc20 or a scrambled control siRNA were immunoblotted with the NeuroD2, Cdc20, or ERK1/2 antibody. (F) Knockdown of NeuroD2 by two different siRNAs (U6/nd2 or U6/nd2.2) in granule neurons led to a greater than 100% increase in synapsin cluster density ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). (G) Expression of NeuroD2-RES, but not NeuroD2-WT, suppressed synapsin clustering in the background of NeuroD2 RNAi ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). (H and I) NeuroD2 knockdown increased the density of synapsin/FM4-64 ($P < 0.01$, t test, $n = 3$) and synapsin/PSD-95 ($P < 0.05$, t test, $n = 3$) coclusters. (J) NeuroD2 knockdown increased the density of Munc13 clusters ($P < 0.001$, t test, $n = 3$). (K) Expression of ND2-DBM suppressed synapsin clustering ($P < 0.01$, t test, $n = 3$). (L) Knockdown of Cdc20 reduced synapsin clustering ($P < 0.05$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$), but did not reduce synapsin clustering in the background of NeuroD2 RNAi.

Fig. 3. The *NeuroD2* target gene, Complexin II, inhibits presynaptic differentiation. **(A)** Granule neurons were transfected with the GFP-synapsin expression plasmid together with the control U6 or U6/*cplx2* RNAi plasmid and an expression plasmid encoding Cplx2-WT, Cplx2-RES, or a control vector. Cplx2 knockdown increased synapsin clustering, and expression of Cplx2-RES but not Cplx2-WT reduced synapsin cluster density in the background of Cplx2 RNAi ($P < 0.01$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). **(B and C)** Cplx2 knockdown increased the density of synapsin/PSD-95 and synapsin/FM4-64 coclusters. For quantification, see fig. S8, C and D. **(D)** Cplx2 knockdown induced Munc13 clustering ($P < 0.001$, t test, $n = 3$). **(E)** Expression of exogenous Cplx2 suppressed the *NeuroD2* RNAi-induced increase in synapsin clustering ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). **(F)** Simultaneous knockdown of Cplx2 and *NeuroD2* did not increase synapsin density to a greater extent than with knockdown of each gene alone. **(G)** Knockdown of Cplx2 reversed the *Cdc20* RNAi-induced loss of synapsin clusters ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). Scale bars, 20 μ m.



We assessed the role of *Cdc20*-APC in the regulation of *NeuroD2* protein abundance in neurons. *NeuroD2* formed a physical complex with *Cdc20* in cells, but a *NeuroD2* protein in which the D-box was mutated (*ND2-DBM*) failed to associate with *Cdc20* (Fig. 2D and fig. S5C). Endogenous *NeuroD2* levels increased upon *Cdc20* knockdown in neurons (Fig. 2E). Thus, *Cdc20*-APC controls the abundance of *NeuroD2* protein in neurons.

The identification of *NeuroD2* as a target of *Cdc20*-APC in neurons would predict that *NeuroD2* suppresses synapsin clustering. Consistent with this prediction, *NeuroD2* knockdown increased the density of synapsin clusters in granule neurons by a factor of more than two (Fig. 2F and fig. S5, D and E). Expression of an RNAi-resistant rescue form of *NeuroD2* (*NeuroD2-RES*), but not *NeuroD2-WT*, reversed the *NeuroD2* RNAi-induced increase in synapsin cluster density in neurons (Fig. 2G and fig. S5F). *NeuroD2* knockdown also increased synapsin cluster density in parallel fiber axons in cerebellar slices (fig. S6A). In other experiments, *NeuroD2* knockdown in granule neurons increased the density of synapsin/FM4-64 and synapsin/Syt1 coclusters by a factor of more than two (Fig. 2H and fig. S6, B to D). Further, the *NeuroD2* knockdown-induced synapsin clusters were apposed to PSD-95 (Fig. 2I and fig. S6E). *NeuroD2* knockdown also increased clustering of the active zone protein Munc13 in

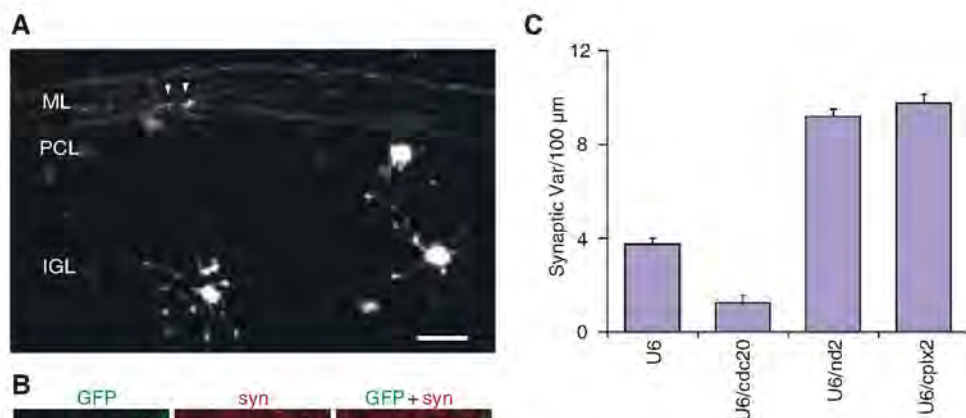


Fig. 4. The *Cdc20*-APC ubiquitin signaling pathway regulates presynaptic axonal differentiation in vivo. **(A)** A representative image is shown of a cerebellum from a P12 rat pup electroporated with the U6-cmvGFP expression plasmid 9 days earlier. Granule neurons have descended to the IGL and axonal parallel fibers reside in the ML. Arrowheads denote varicose structures along the parallel fibers that represent presynaptic sites (12). **(B)** Cerebellar sections as prepared in (A) were immunostained with the GFP and synapsin (top) or PSD-95 (bottom) antibodies. **(C)** *Cdc20* knockdown reduced, whereas *NeuroD2* and *Cplx2* knockdown increased, the density of presynaptic varicosities in the cerebellar cortex in vivo ($P < 0.001$, ANOVA followed by Fisher's PLSD post hoc test, $n = 3$). Scale bars, 20 μ m.

granule neurons (Fig. 2J and fig. S6F). Thus, NeuroD2 knockdown increases the density of functionally active sites of presynaptic differentiation.

In gain-of-function analyses, expression of the mutant NeuroD2 protein ND2-DBM in neurons reduced the density of presynaptic sites (Fig. 2K and fig. S7, A to C). In epistasis analyses, NeuroD2 knockdown suppressed the effect of Cdc20 knockdown on presynaptic differentiation (Fig. 2L and fig. S7, D and E). These results suggest that Cdc20-APC promotes the formation of functional presynaptic axonal sites through NeuroD2 degradation in neurons.

Among the reported NeuroD2 targets is the gene encoding Complexin II (Cplx2), a protein that modulates synaptic vesicle fusion to the presynaptic membrane in invertebrates (9, 10). We asked if Cplx2 might mediate NeuroD2-dependent control of presynaptic differentiation. Knockdown of Cplx2 robustly increased the density of synapsin clusters in granule neurons, and this phenotype was reversed by expression of Cplx2 encoded by an RNAi-resistant cDNA (Cplx2-RES) but not wild-type cDNA (Cplx2-WT) (Fig. 3A and fig. S8, A and B). Cplx2 knockdown also increased the density of functionally active presynaptic axonal sites in granule neurons (Fig. 3, B to D, and fig. S8, C to F). Finally, Cplx2 knockdown increased synapsin cluster density in granule neuron parallel fiber axons in the cerebellar cortex in slices (fig. S8G). Thus, Cplx2 knockdown phenocopies the effect of NeuroD2 knockdown on presynaptic differentiation.

In epistasis analyses, expression of exogenous Cplx2 in granule neurons reduced the density of functionally active presynaptic sites induced by NeuroD2 RNAi (Fig. 3E and fig. S9, A and B). Further, although knockdown of NeuroD2 or Cplx2 each stimulated presynaptic differentiation, the combination of NeuroD2 and Cplx2 knockdown together did not additively increase the density of presynaptic sites (Fig. 3F and fig. S9, C and D). In other experiments, knockdown of Cplx2 reversed Cdc20 RNAi-induced suppression of presynaptic differentiation (Fig. 3G and fig. S9, E and F). Collectively, these results suggest that Cplx2 mediates the ability of NeuroD2 to suppress presynaptic differentiation and acts as a downstream component of the Cdc20-APC pathway in the control of presynaptic development.

We next determined the role of the Cdc20-APC ubiquitin signaling pathway in presynaptic differentiation in the developing organism. To visualize the morphogenesis of presynaptic axonal differentiation in vivo, we expressed GFP in the cerebellar cortex in rat pups using an electroporation method (3, 5, 11). These analyses revealed granule neuron cell bodies in the internal granule layer (IGL) and their parallel fiber axons in the molecular layer (ML) (Fig. 4A). Parallel fiber axons displayed varicosities along the axon (Fig. 4B), which colocalized with punctate synapsin immunoreactivity and were apposed to punctate PSD-95, a marker of postsynaptic structures (Fig. 4B). These observations indicate that parallel fiber axon varicosities represent sites of presynaptic axonal differentiation in vivo.

Using electroporation, we next induced knockdown of Cdc20, NeuroD2, or Cplx2 in rat pups. Cdc20 knockdown reduced the density of presynaptic parallel fiber varicosities in the cerebellar cortex in vivo. In contrast, knockdown of NeuroD2 or Cplx2 in rat pups increased the density of presynaptic varicosities in the cerebellar cortex (Fig. 4C and fig. S10). These results suggest that the Cdc20-APC ubiquitin signaling pathway cell-autonomously regulates presynaptic axonal differentiation in the developing brain in vivo.

We have identified a Cdc20-APC ubiquitin ligase signaling pathway that orchestrates presynaptic development and hence the establishment of neuronal circuitry in the brain (see model in fig. S11). APC function in cell cycle control has been the subject of intense scrutiny (8). Whereas Cdh1-APC operates in late mitosis and G1 phase of the cell cycle, Cdc20-APC controls the transition of the cell cycle through early mitosis (8). In the control of axon morphogenesis in postmitotic neurons, Cdh1-APC appears to control axon growth and patterning (3), whereas Cdc20-APC operates at a later developmental stage to promote presynaptic axonal differentiation. Thus, in an analogous manner to the temporal control of the cell cycle, the APC in concert with its two different coactivators, Cdh1 and Cdc20, appears to govern distinct temporal phases of axon differentiation in postmitotic neurons in the brain.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5952/575/DC1

Materials and Methods

Figs. S1 to S13

References

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Metagenome of a Versatile Chemolithoautotroph from Expanding Oceanic Dead Zones

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Oxygen minimum zones, also known as oceanic "dead zones," are widespread oceanographic features currently expanding because of global warming. Although inhospitable to metazoan life, they support a cryptic microbiota whose metabolic activities affect nutrient and trace gas cycling within the global ocean. Here, we report metagenomic analyses of a ubiquitous and abundant but uncultivated oxygen minimum zone microbe (SUP05) related to chemoautotrophic gill symbionts of deep-sea clams and mussels. The SUP05 metagenome harbors a versatile repertoire of genes mediating autotrophic carbon assimilation, sulfur oxidation, and nitrate respiration responsive to a wide range of water-column redox states. Our analysis provides a genomic foundation for understanding the ecological and biogeochemical role of pelagic SUP05 in oxygen-deficient oceanic waters and its potential sensitivity to environmental changes.

Dissolved oxygen (O_2) concentration is a critical determinant of marine ecosystem structure and function. O_2 deficiency results in habitat compression and reduced productivity for aerobic organisms, with concomitant expansion of conditions favoring chemolithotrophic energy metabolism (1), which results in nitrogen loss and production of climate-active trace gases such as nitrous oxide (N_2O) and methane (CH_4)

(2, 3). Extensive oxygen minimum zones (OMZs), defined by O_2 concentrations $< 20 \mu M$, are found throughout the eastern North Pacific (ENP), eastern South Pacific (ESP), northern Indian Ocean, and southwest African shelf waters (3). Moreover, climate change-induced expansion and intensification of OMZs is occurring globally, with potentially deleterious effects on oceanic nitrogen cycling and carbon sequestration (1, 4–6).

Although the extent of oxygen deficiency varies among OMZs, taxonomic surveys reveal conserved patterns of microbial community composition (7–9). In all sites examined, small subunit ribosomal RNA (SSU rRNA) gene libraries are enriched with sequences affiliated with chemotrophic gill symbionts of deep-sea clams and mussels (ESP-OMZ-sequence-accumulation-1 (EOSA-1) in the ESP and gamma-proteobacterial sulfur oxidizer (GSO) cluster in African shelf waters) (9–11). Phylogenetic analyses indicate that the GSO-EOSA-1 complex consists of two closely related, cooccurring and uncultivated lineages, ARCTIC96BD-19 (12) and SUP05 (13), with the latter encompassing the symbionts (Fig. 1, A and B). Blooming SUP05 populations

have recently been implicated in chemolithotrophic sulfide (H_2S) oxidation coupled to nitrate (NO_3^-) reduction in African shelf waters (10). Both ARCTIC96BD-19 and SUP05 populations are also found in nonsulfidic waters of the ENP and ESP, which suggests that they can adopt alternative modes of energy metabolism. Given the potential importance of ARCTIC96BD-19 and SUP05 to carbon, nitrogen, and sulfur cycling in OMZs, a deeper understanding of the metabolic capabilities of these lineages is needed to constrain their respective ecological and biogeochemical roles.

Saanich Inlet, British Columbia, is a seasonally anoxic fjord characterized by an annual stratification and deep-water renewal cycle (14), resulting in large water-column redox gradients and high rates of trace gas production and consumption (fig. S1, A to C). Previously, we identified pelagic SUP05 in the Saanich Inlet water column, representing up to 37% of total bacteria (table S1) (11). Further examination of SUP05 SSU rRNA gene copy number during seasonal stratification revealed blooming populations below the oxycline, reaching up to 4.75×10^5 copies per ml in regions of H_2S and NO_3^- depletion (15) (fig. S2). High-resolution SSU rRNA gene sur-

veys revealed two SUP05 phylotypes, SI-1 and SI-2 (Figs. 1A and 2A), differing by ~4% nucleotide identity. Although SI-1 dominated suboxic waters year round, SI-2 was less common, and transiently increased during deep-water renewal events, which indicated ecologically differentiated populations (Fig. 2A). Here we analyzed 16 bi-directionally end-sequenced fosmid libraries constructed from environmental DNA samples spanning oxic to anoxic waters over the seasonal stratification and deep-water renewal cycle in order to reconstruct the metabolic potential of uncultivated pelagic SUP05 populations (fig. S1D).

To identify SUP05-specific scaffolds, fosmid paired-end sequences were assembled and binned on the basis of shared sequence similarity to symbiont reference genomes (16, 17) and analysis of intrinsic oligonucleotide composition patterns (fig. S3). Nineteen scaffolds encompassing 1.16 million base pairs of SUP05 DNA (SUP05 metagenome) were identified (table S2) and taxonomically verified (table S3). Consistent with SSU rRNA gene survey data, a majority of fosmid paired-end sequences within SUP05 scaffolds were derived from samples exhibiting elevated SI-1 phylotype abundance (Fig. 2B). Of

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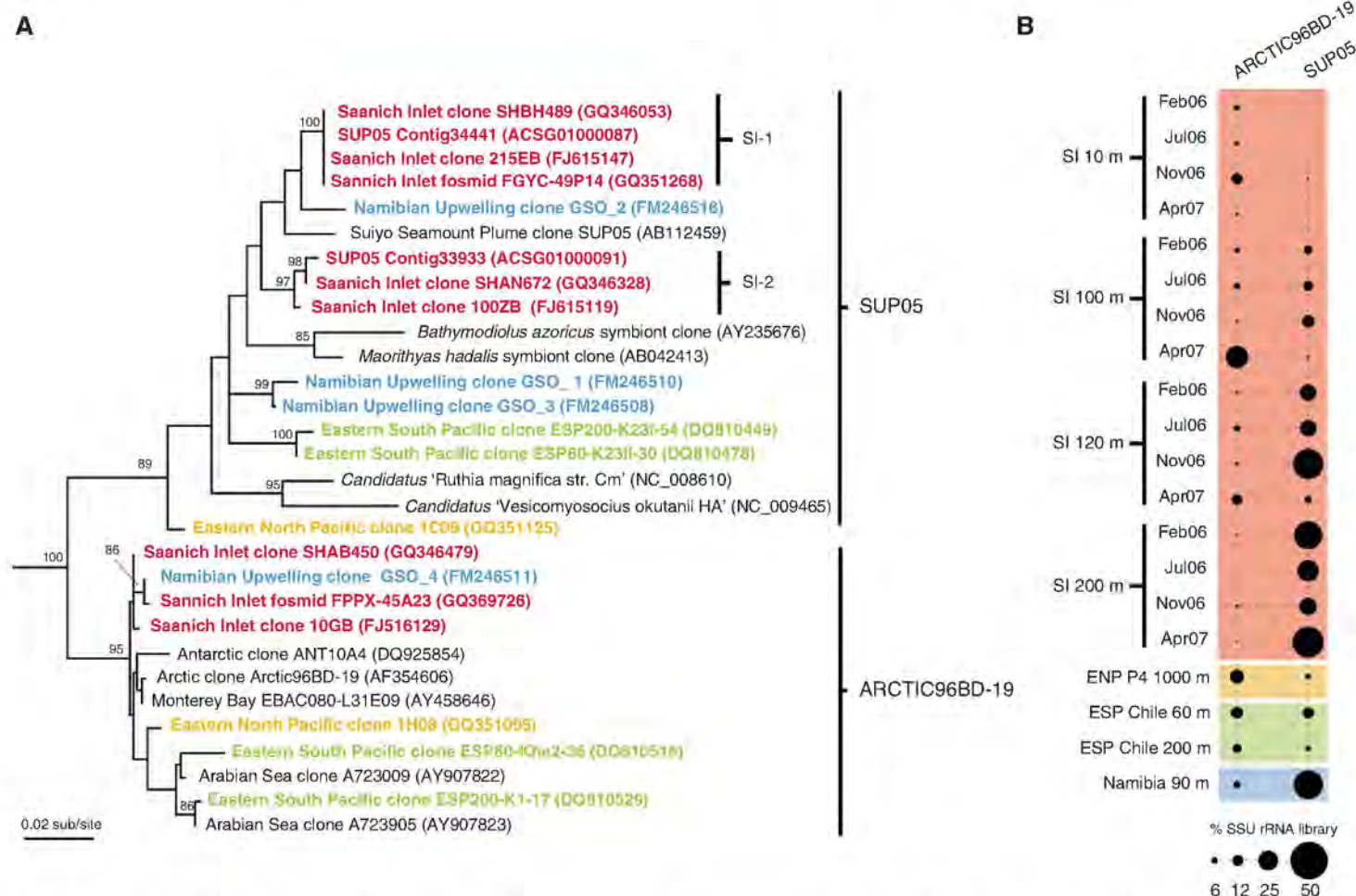


Fig. 1. (A) Phylogenetic tree of ARCTIC96BD-19 and SUP05 lineages based on comparative SSU rRNA gene analysis. The tree was inferred using maximum likelihood implemented in PHYML. (B) Relative abundance of

ARCTIC96BD-19 and SUP05 SSU rRNA sequences recovered from Saanich Inlet (SI), eastern North Pacific (ENP), eastern South Pacific (ESP) (9), and southwest African shelf waters (Namibia) (10).

Fig. 2. (A) Phylotype abundance of SUP05 SI-1 (black circles) and SI-2 (white circles) based on recovery of SSU rRNA gene sequences in PCR-generated clone libraries. (B) Mean depth of coverage of the SUP05 metagenome plotted over the Saanich Inlet nitrate profile during the 2006–07 season. Sample dates and depths are noted on the x and y axes, respectively.

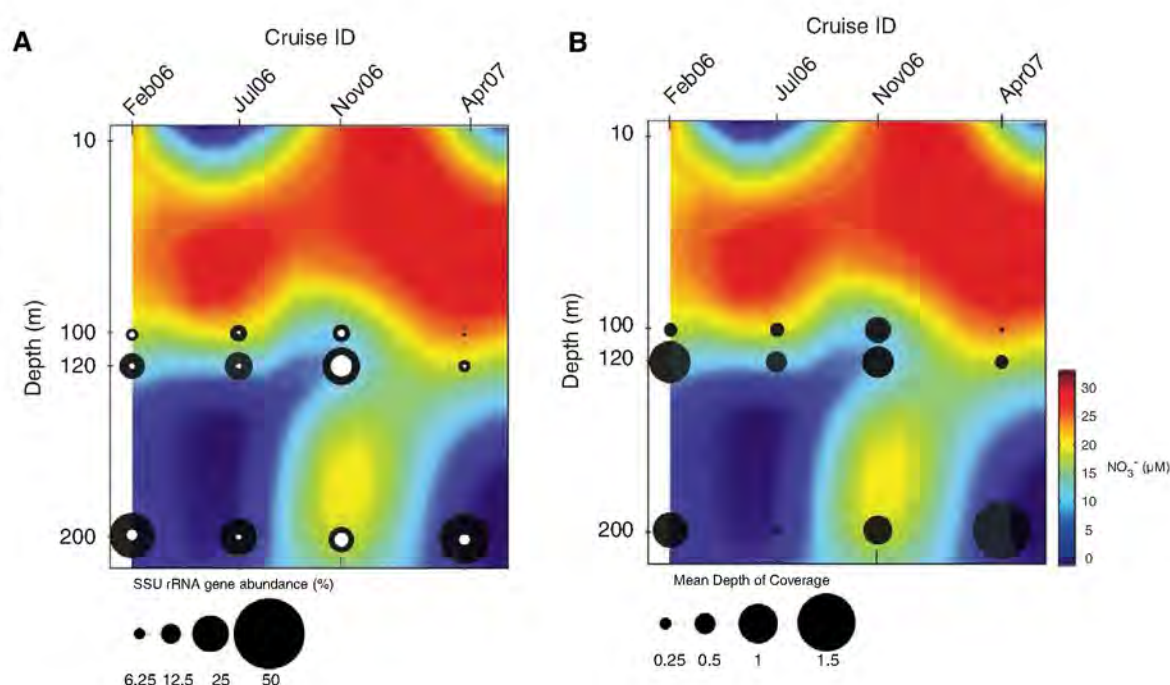
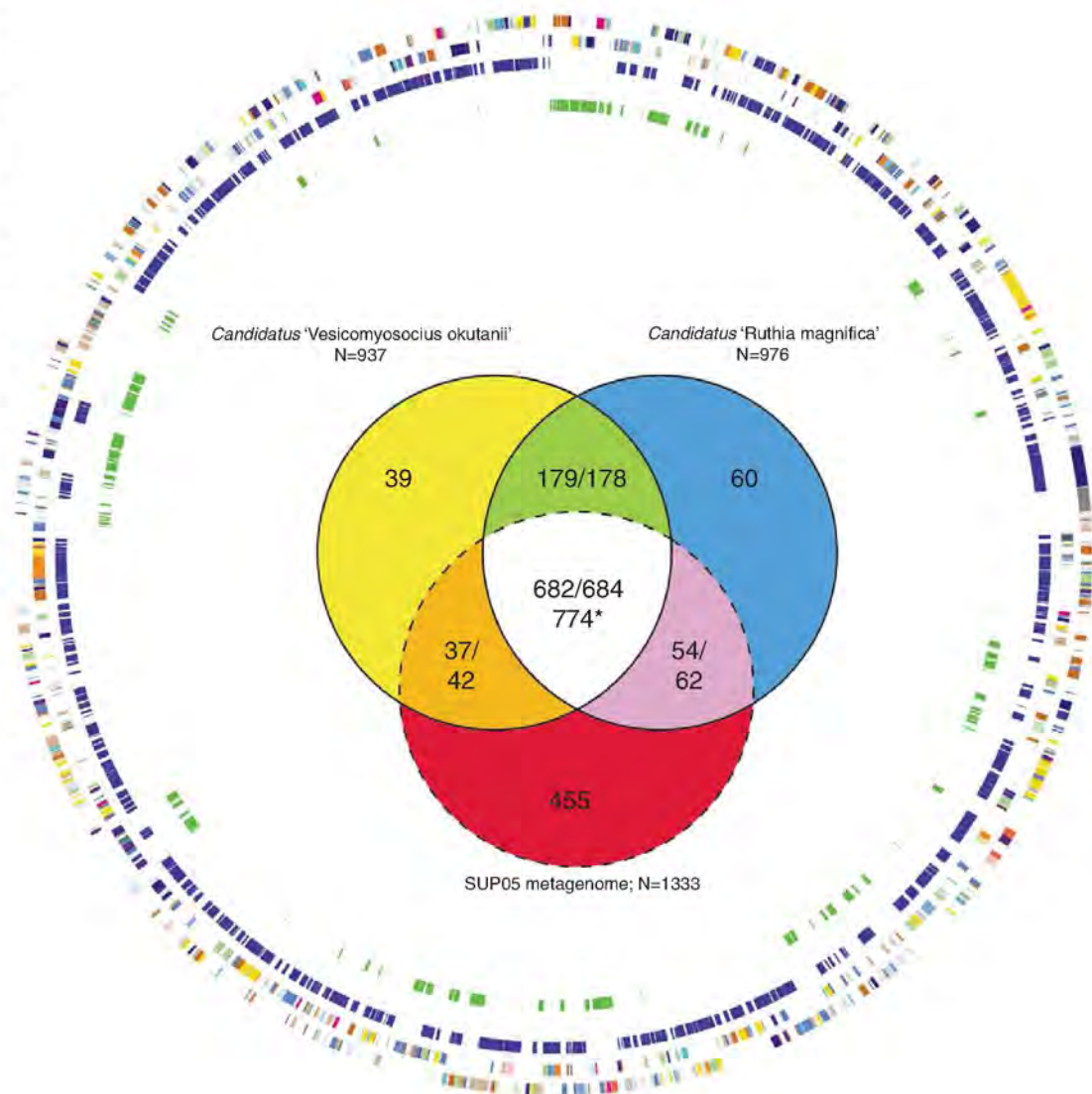


Fig. 3. Gene content comparison between SUP05 metagenome and symbiont reference genomes. Nested circles from outermost to innermost represent the following: (i and ii) Functional predictions based on clusters of orthologous groups (COG) on the forward and reverse strands of the *R. magnifica* reference genome (see fig. S4 for COG color code designation). (iii) Conservation of gene content and (iv) genes conserved in symbionts, but absent from the SUP05 metagenome. (Inset) Venn diagram depicts predicted gene distribution among SUP05 metagenome and symbiont reference genomes. Values correspond to the number of shared genes among overlapping genomes, with each genome used as the original query. The dotted line represents the open-genome configuration of the SUP05 metagenome. *The discrepancy in core size when SUP05 metagenome is used as query (774) compared with symbionts (~683) reflects gene content redundancy in the metagenome assembly.



861 genes shared between symbiont reference genomes, 80% were conserved in the SUP05 metagenome (supporting online material text, Fig. 3 and fig. S4). Many of these genes are predicted to mediate informational processing steps, particularly translation, although a significant fraction function in carbon, sulfur, amino acid, and coenzyme metabolism (figs. S5 and S6). Approximately 35% of gene content predicted in the SUP05 metagenome was unique, including genes implicated in DNA uptake and repair, denitrification, and adaptive or stress responses (15).

Similar to symbiotic counterparts, the SUP05 metagenome harbors genes mediating the Calvin-Benson-Bassham (CBB) cycle for autotrophic carbon assimilation, including a single form II ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) gene, which both implicate SUP05 in chemosynthetic carbon fixation (18). In addition, a gene encoding β -class carbonic anhydrase, a potential CO_2 -concentrating mechanism, was also identified. The repertoire of genes required for converting fixed carbon to hexose and ribose sugars via gluconeogenesis and the nonoxidative branch of the pentose phosphate pathway were identified, along with the majority of tricarboxylic acid (TCA) cycle components (fig. S6). However, we did not recover genes for enzymes mediating the interconversion of succinyl-CoA and 2-oxoglutarate (fig. S6), which indicated that SUP05 is potentially an obligate autotroph as posited earlier for clam symbionts (16, 17).

From the standpoint of energy metabolism, the SUP05 metagenome harbors a diverse repertoire of genes involved in chemolithotrophic oxidation of reduced sulfur compounds. We found genes encoding flavocytochrome c sulfide dehydrogenase (*fccAB*) and sulfide quinone oxidoreductase (*sqr*), mediating oxidation of H_2S to elemental sulfur (S^0) (fig. S6). The presence of two enzymatic complexes may facilitate sulfur-based energy metabolism under variable sulfide regimes. In addition, dissimilatory sulfite reduc-

tase subunits (*dsrAB*), adenosine 5'-phosphosulfate (APS) reductase (*apr*), and adenosine 5'-triphosphate (ATP) sulfurylase (*sat*) mediating complete oxidation of S^0 to sulfate, and the Sox pathway (*soxABXYZ*) for thiosulfate ($\text{S}_2\text{O}_3^{2-}$) oxidation (fig. S6) (19) were conserved between pelagic SUP05 and symbiont reference genomes. The capacity to obtain electrons from $\text{S}_2\text{O}_3^{2-}$ may be of ecological relevance, given that mixing of sulfidic and oxygenated water masses results in $\text{S}_2\text{O}_3^{2-}$ accumulation owing to chemical oxidation of H_2S (20, 21). Moreover, the apparent absence of *soxCD* sulfur dehydrogenase genes indicates the capacity to store S^0 , which can be subsequently oxidized via the reverse dissimilatory sulfate reduction (DSR) pathway and thereby provisions SUP05 in the absence of ambient reductant (22). Indeed, *soxCD* homologs are also absent from symbiont reference genomes, and colloidal sulfur globule formation has been associated with a subset of clam symbionts (23).

Although symbiont reference genomes harbor multiple aerobic respiratory complexes (16, 17), none were recovered in the SUP05 metagenome, consistent with a facultative or strictly anaerobic life-style. Indeed, all enzymatic machinery needed to reduce NO_3^- to nitrous oxide (N_2O), including membrane-bound (*narKK₂GHI*) and periplasmic (*napFBAHGD*) dissimilatory nitrate reductases, which potentially operate under high and low NO_3^- conditions, respectively (24, 25); copper-containing nitrite reductase (*nirK*); and N_2O forming nitric oxide reductase (*norCB*) (Fig. 4 and fig. S6), were identified, which mechanistically implicated pelagic SUP05 in biological nitrogen loss from oxygen-deficient oceanic waters. Moreover, the genomic colocalization of sulfur oxidation and denitrification genes in close proximity to Crp/Fnr transcriptional regulators (Fig. 4) may allow coordinated gene expression in response to changing redox states or nutrient limitation (24, 26, 27).

We identified more than 10 putative toxin-antitoxin (TA) modules in the SUP05 metage-

nome, which indicated a highly regulated stress response (table S4). TA modules consist of a stable toxin and a labile antitoxin and are commonly associated with environmental bacteria, where they control induction of reversible cellular stasis (28). Of specific interest is a TA module of the RelE superfamily identified within an operon encoding molybdopterin-guanine dinucleotide synthase (*mobA*) (Fig. 4). The product of MobA, molybdopterin-guanine dinucleotide (MGD), is an essential cofactor for all described classes of nitrate reductase (29), and therefore, *mobA* expression is integral to denitrification. In this regard, the integration of a TA system into a denitrification regulon may allow SUP05 to persist during periods of extreme NO_3^- limitation, analogous to other forms of nutritional stress response (e.g., amino acid starvation in *Escherichia coli*) (30).

As the number of studies surveying OMZ community structure increases, the ubiquity and abundance of SUP05 becomes apparent. Analysis of the SUP05 metagenome, together with water-column disposition of pelagic SUP05 with respect to H_2S and NO_3^- gradients, resolves a chemolithoautotrophic metabolism based on oxidation of reduced sulfur compounds with NO_3^- through multiple and highly regulated bioenergetic routes. Although pelagic distribution of SUP05 in Saanich Inlet and African shelf waters is predicated on the basis of vertical H_2S and NO_3^- gradients, the presence of this lineage in nonsulfidic OMZs (e.g., ENP and ESP) emphasizes the need to more fully resolve the chemical speciation of sulfur compounds with respect to SUP05 physiology and population structure. Moreover, the contribution of SUP05-mediated denitrification to the present unbalanced nitrogen cycle and the potential interplay with anaerobic ammonia-oxidizing bacteria within sulfidic and nonsulfidic OMZs remains to be investigated.

Paradoxically, as "dark" primary producers, blooming SUP05 populations have the potential to fix CO_2 while simultaneously producing N_2O .

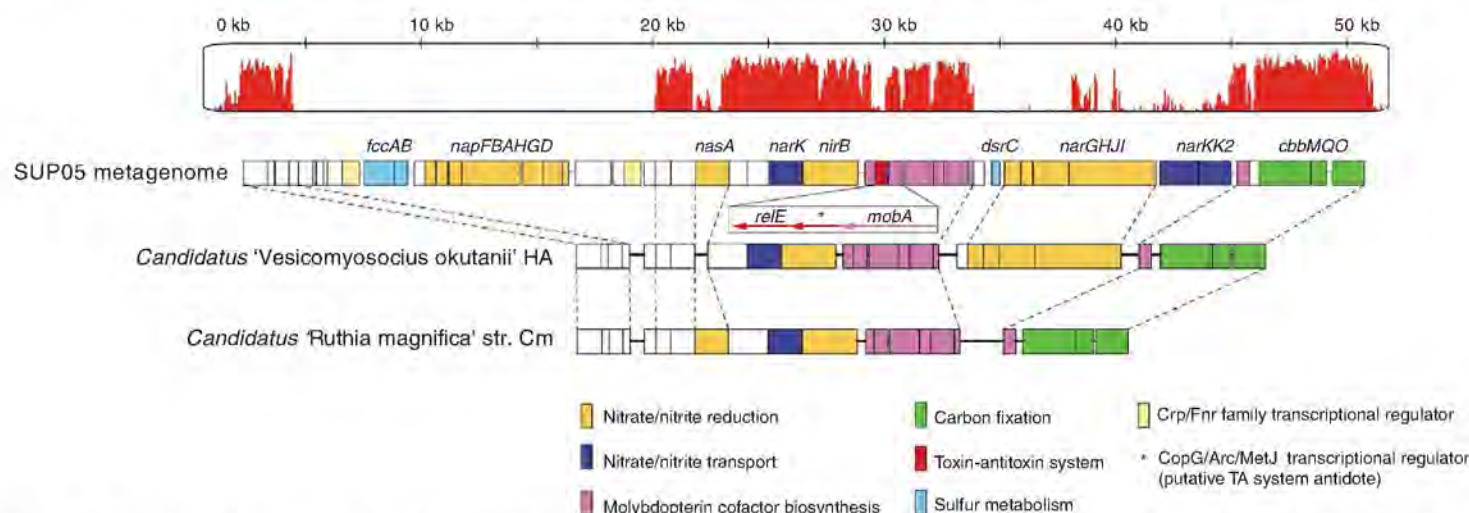


Fig. 4. Alignment of an ungapped region of a SUP05 metagenomic scaffold, encoding genes involved in nitrate and sulfur metabolism, with the corresponding genomic regions of symbiont reference genomes. The

height of red bars corresponds to nucleotide similarity over conserved genomic regions. Proper scaffold assembly across this region was verified by full-length sequencing of two overlapping fosmids.

As habitat range increases with OMZ expansion and intensification, this role will only become more visible and significant. Therefore, the SUP05 metagenome provides a functional template for analysis of gene expression in relation to climatologically relevant biogeochemical transformations within oxygen-deficient oceanic waters. This information should prove useful in the development of monitoring tools to assess microbial community responses to OMZ expansion and intensification.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S7

Tables S1 to S4

References

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Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines

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The pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the skin disease chytridiomycosis, is one of the few highly virulent fungi in vertebrates and has been implicated in worldwide amphibian declines. However, the mechanism by which *Bd* causes death has not been determined. We show that *Bd* infection is associated with pathophysiological changes that lead to mortality in green tree frogs (*Litoria caerulea*). In diseased individuals, electrolyte transport across the epidermis was inhibited by >50%, plasma sodium and potassium concentrations were respectively reduced by ~20% and ~50%, and asystolic cardiac arrest resulted in death. Because the skin is critical in maintaining amphibian homeostasis, disruption to cutaneous function may be the mechanism by which *Bd* produces morbidity and mortality across a wide range of phylogenetically distant amphibian taxa.

Infectious disease can cause population declines (1), and potentially extinctions (2), if multiple variables create favorable conditions for severe outbreaks. A striking example is the global loss of amphibians due to chytridiomycosis (1, 3, 4). Despite an initial reluctance to accept disease as a direct cause of declines (5), *Batrachochytrium dendrobatidis* (*Bd*) is now recognized for its ability to spread rapidly through amphibian populations (6, 7), infect numerous species (1, 6), cause high rates of mortality (6, 8),

and persist even at low host densities (7, 9). These disease characteristics render population recovery from chytridiomycosis especially difficult and provide strong evidence for disease-induced extinctions (2, 8, 10). However, the mechanism by which *Bd* kills amphibians is unknown.

The pathogenesis of chytridiomycosis has been difficult to determine because cutaneous fungal infections are rarely fatal without other predisposing factors (11). Furthermore, *Bd* is in

a phylum of fungi not previously known as pathogens of vertebrates (12), it is confined to the superficial layers of the epidermis (2, 13) with minimal host reaction to infection (13, 14), and no consistent pathological changes in internal organs of diseased amphibians are detectable with light microscopy (3). Differential expression of peptidase genes suggests that *Bd* pathogenicity may have a genetic basis (15), but determining the proximate cause of death has been inherently challenging because multiple physiological systems shut down before death.

Amphibian skin is unique among terrestrial vertebrates because it is actively involved in the exchange of respiratory gases, water, and electrolytes (16–19). Because of the role of amphibian skin in maintaining osmotic balance, other studies have suggested that *Bd* might disrupt cutaneous osmoregulation (3, 20). To test this hypothesis, we tracked the development of *Bd* infections in green tree frogs (*Litoria caerulea*), which are susceptible to chytridiomycosis in

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laboratory experiments (21), with polymerase chain reaction (PCR) analysis and histopathology on skin biopsies. Clinical signs of disease and mortality occurred in *L. caerulea* individuals with the highest burdens of *Bd* (Fig. 1) and with greatest histopathological changes in the epidermis (fig. S2). We measured epidermal electrolyte

transport in isolated skin preparations, monitored changes in blood and urine biochemical parameters, and monitored cardiac electrical activity with implanted biotransmitters in control, acclimated, and clinically diseased frogs.

To maintain osmotic balance, amphibians must sustain a hyperosmotic internal environment

relative to the external environment (16–19). This is accomplished by tight regulation of electrolyte absorption across the epidermis, involving sodium channels and Na^+/K^+ pumps (16–19). Basal electrolyte transport across the skin, measured as equivalent short-circuit current in electrophysiological tests, was lower in skin samples from diseased frogs than in those from control frogs (Fig. 2A) and was accompanied by reduced transepithelial resistance (Fig. 2B). We estimated sodium absorption as the component of the short-circuit current that was blocked by amiloride (Fig. 2E), a specific inhibitor of the sodium channel (22). The residual short-circuit current in the presence of amiloride did not differ between skin samples from diseased and control frogs (Fig. 2F), indicating that *Bd* infection predominantly inhibits sodium absorption. Additionally, responses to carbachol (Fig. 3G), which activates chloride secretion in frog skin (23), and to noradrenaline (Fig. 3H), which stimulates sodium absorption and chloride secretion (24), indicated reduced sodium and chloride channel activity in epidermis from clinically diseased frogs. The biochemical mechanisms of epidermal channel disruption are unknown but could be due

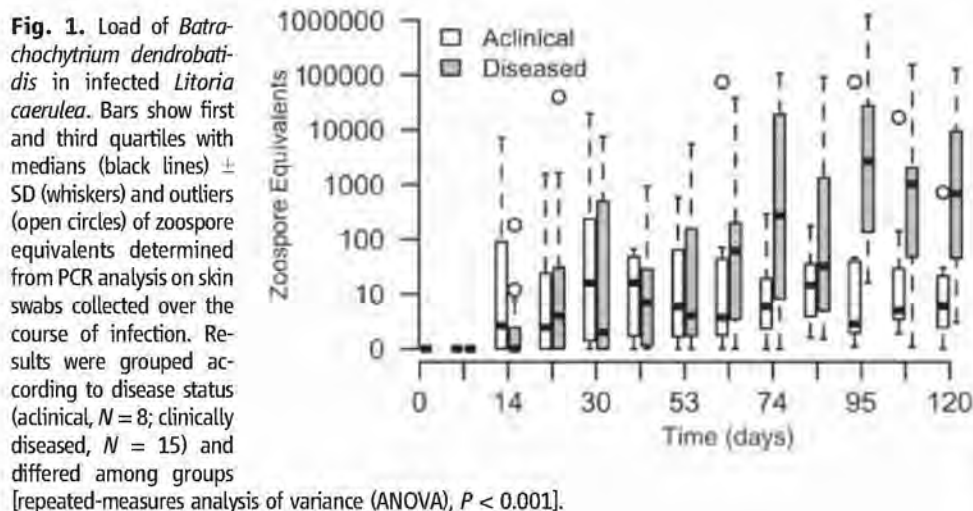


Fig. 1. Load of *Batrachochytrium dendrobatidis* in infected *Litoria caerulea*. Bars show first and third quartiles with medians (black lines) $\pm$ SD (whiskers) and outliers (open circles) of zoospore equivalents determined from PCR analysis on skin swabs collected over the course of infection. Results were grouped according to disease status (acclimated, $N = 8$; clinically diseased, $N = 15$) and differed among groups [repeated-measures analysis of variance (ANOVA), $P < 0.001$].

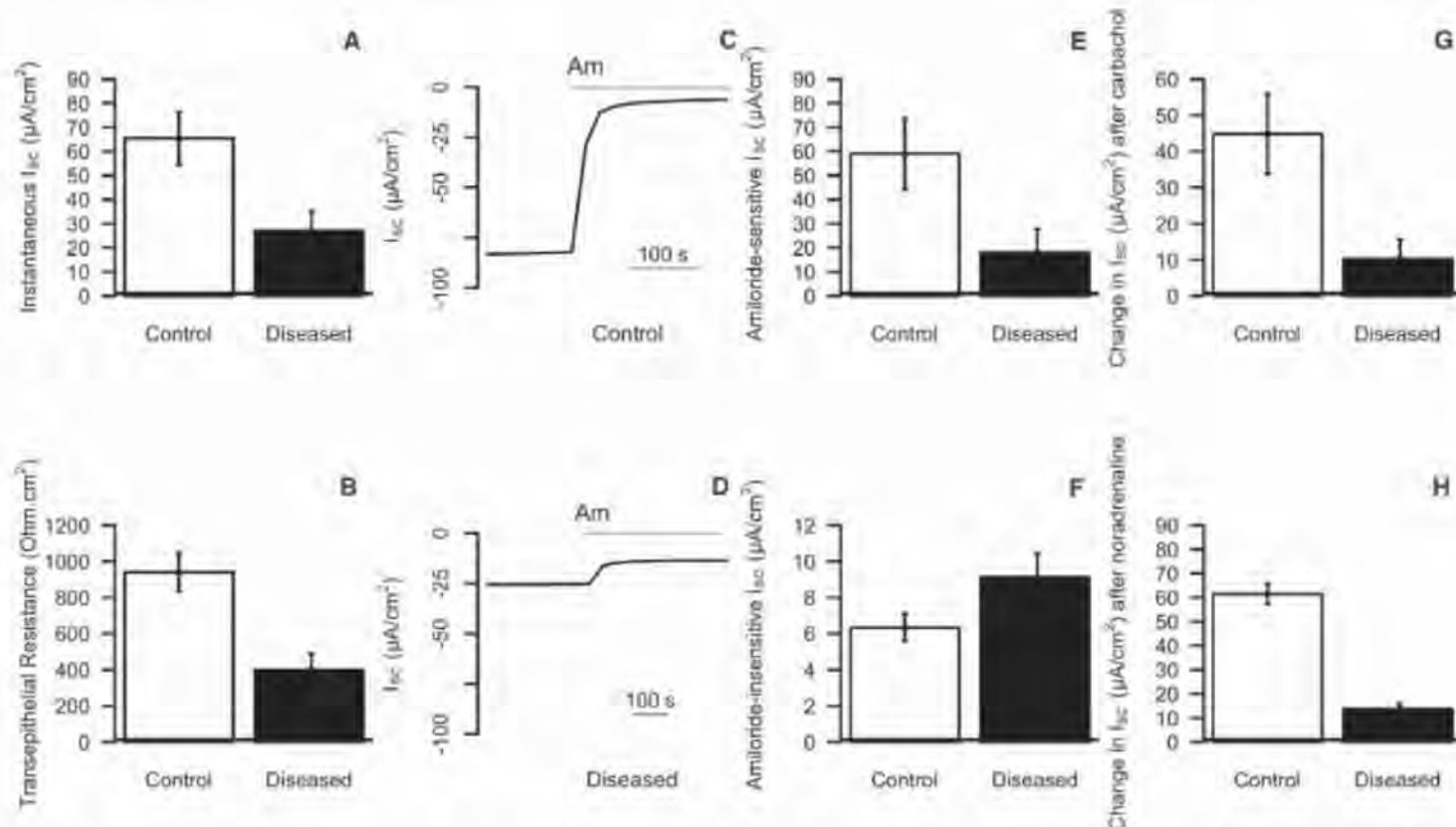


Fig. 2. Electrophysiological measurements of electrolyte transport across ventral skin samples from *L. caerulea* infected with *B. dendrobatidis*. Data in bar graphs show means ($\pm$ SEM) from control ($N = 7$) and clinically diseased ($N = 7$) *L. caerulea*. (A and B) Instantaneous short-circuit current and transepithelial resistance were reduced in skin samples from clinically diseased *L. caerulea* [(A), Student's t test, $P = 0.009$; (B), $P = 0.006$]. (C and D) Original tracings show short-circuit current before and after blocking of sodium absorption with amiloride in skin samples from a control (C) and a

clinically diseased *L. caerulea* (D). (E and F) The component of the amiloride-sensitive current (E) differed (Student's t test, $P = 0.038$) between the two groups, but amiloride-insensitive (residual) current (F) did not (Student's t test, $P = 0.79$), indicating inhibition of sodium absorption in clinically diseased *L. caerulea*. (G and H) The change in transepithelial short-circuit current was reduced in clinically diseased *L. caerulea* after treatment with carbachol [(G), Student's t test, $P = 0.015$] or noradrenaline [(H), Student's t test, $P = 0.001$].

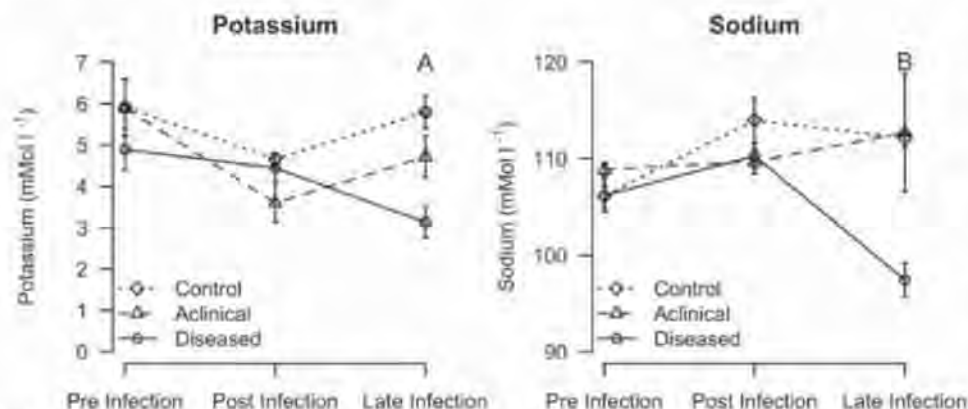


Fig. 3. Blood plasma potassium and sodium concentrations in *L. caerulea*. Blood samples were collected from infected *L. caerulea* (acinical, $N = 7$; clinically diseased, $N = 11$; uninfected control, $N = 7$) on three sample occasions: 20 days before exposure (Pre Infection), 30 days after exposure (Post Infection), and 60 to 123 days after exposure when clinical signs of disease were obvious (Late Infection). (A and B) Data show mean ($\pm$ SEM) concentrations of plasma potassium [(A), repeated-measures ANOVA, $P = 0.028$] and plasma sodium [(B), repeated-measures ANOVA, $P = 0.002$].

to release of a fungal toxin or direct damage to infected host cells. Nonetheless, these results show that *Bd* compromises electrolyte transport, and thus osmoregulatory function, in the skin of infected *L. caerulea*.

We tested multiple blood and urine parameters as markers of organ function and general health (tables S1 and S2) and observed the greatest and most consistent changes in plasma electrolyte concentrations. Plasma sodium and potassium concentrations were significantly reduced as a result of disease when assessed according to clinical status (Fig. 3). Significant negative correlations existed between intensity of infection (*Bd* load) and the change in plasma sodium, potassium, and calcium concentrations (Pearson correlations: sodium, $r = -0.64$, $P = 0.001$, $N = 23$; potassium, $r = -0.47$, $P = 0.03$, $N = 22$; calcium, $r = -0.44$, $P = 0.04$, $N = 22$). None of the additional parameters changed significantly (tables S1 and S2). We observed no significant decrease in body masses or increases in albumin and total protein concentrations in frogs with chytridiomycosis (table S1), which suggests that there was no change in water volume. Because body mass did not change in diseased frogs, the observed osmotic imbalance most likely resulted from electrolyte loss rather than dilution caused by water uptake.

Several hours before death, the cardiac electrical activity of severely diseased frogs resembled patterns associated with cardiac standstill, also known as asystolic or bradyasystolic cardiac arrest, in other organisms including humans (Fig. 4) (25). Asystolic cardiac arrest occurs when cardiac electrical abnormalities cause contractile failure, reduced blood flow, and ultimately circulatory collapse and death. Although several conditions may initiate this cycle (25), most can be ruled out as factors in this study. Ambient temperatures remained constant, eliminating the possibility of hypothermia. Stable body mass and stable plasma protein and albumin concentrations were evidence against dehydration and hypovolemia (low blood volume). Hypoxia (low blood oxygen) was not completely ruled out, but measurements of peripheral blood oxygen saturation indicated a 20% drop in oxygen only after changes in electrical activity were observed in one individual (26). Furthermore, no changes in blood carbon dioxide were detected in a previous study (20). Thus, shifts in electrolytes and/or acidosis appear to be the most likely cause of cardiac asystolic death.

Although electrolyte imbalance, hypokalemia (low plasma potassium), and hyponatremia (low plasma sodium) could result from depletion via the epidermis or the kidney (17, 18), plasma biochemistry showed no indication of renal damage (table S1). In contrast, the skin, which regulates the bidirectional flux and overall balance of sodium and potassium (17), demonstrated inhibited sodium absorption in the ventral epidermis (Fig. 2), and histology showed degenerative epidermal changes (fig. S2). Thus,

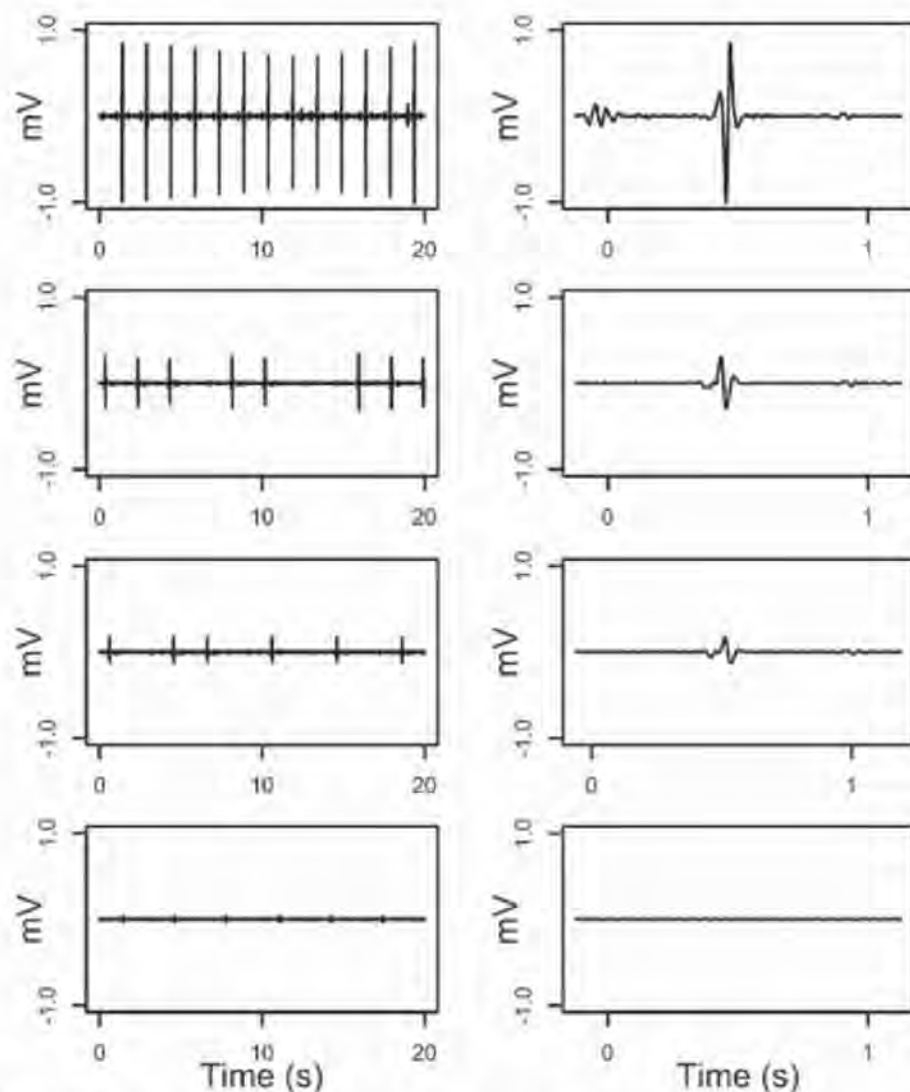


Fig. 4. Original cardiac electrogram tracings from one *L. caerulea* with clinical chytridiomycosis. Heart rate and signal amplitude were determined using 20-s sweeps (left column). Cardiac electrical signal morphology was assessed using 1-s sweeps (right column). Electrograms show cardiac electrical patterns consistent with asystolic cardiac arrest: slowing heart rate, decreasing ventricular depolarization amplitude, and increasing time of ventricular depolarization from (in order from row 1 to row 4) 18, 3, 2, and 0.5 hours before death.

the pathophysiology of chytridiomycosis appears to be disruption to the osmoregulatory functioning of the skin and consequent osmotic imbalance that leads to cardiac standstill.

To test whether treating electrolyte abnormalities would reduce the clinical signs of disease, we administered an oral electrolyte supplement to *L. caerulea* in the terminal stages of infection, when they lost the righting reflex and could no longer correct their body positions (26). Frogs under treatment recovered a normal posture and became more active; one individual recovered sufficiently to climb out of the water onto the container walls, and two individuals were able to jump to avoid capture. These signs of recovery were not observed in any untreated frogs. In addition, treated frogs lived >20 hours longer than untreated frogs [mean time after treatment $\pm$ SEM: treated frogs ($N = 9$), 32 ± 2.8 hours; control frogs ($N = 6$), 10.7 ± 2.2 hours; Student's *t* test, $P < 0.001$]. All treated frogs continued to shed skin and ultimately died from the infection, as expected. It is unlikely that electrolyte treatment could prevent death unless the epidermal damage caused by *Bd* is reversed. Although amphibians can generally tolerate greater electrolyte fluctuations than other terrestrial vertebrates (18), we suggest that depletion of electrolytes, especially potassium, is important in the pathophysiology of chytridiomycosis. Amphibian plasma potassium concentrations are maintained at constant levels across seasons (27), and even moderate hypokalemia is dangerous in humans (28).

Our results support the epidermal dysfunction hypothesis, which suggests that *Bd* disrupts cutaneous osmoregulatory function, leading to electrolyte imbalance and death. The ability of *Bd* to

compromise the epidermis explains how a superficial skin fungus can be fatal to many species of amphibians; their existence depends on the physiological interactions of the skin with the external environment (16–19). Disease outbreaks capable of causing population declines require the alignment of multiple variables, including a life-compromising pathophysiology (1). Resolving the pathogenesis of chytridiomycosis is a key step in understanding this unparalleled pandemic.

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Supporting Online Material

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Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

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Chronic fatigue syndrome (CFS) is a debilitating disease of unknown etiology that is estimated to affect 17 million people worldwide. Studying peripheral blood mononuclear cells (PBMCs) from CFS patients, we identified DNA from a human gammaretrovirus, xenotropic murine leukemia virus-related virus (XMRV), in 68 of 101 patients (67%) as compared to 8 of 218 (3.7%) healthy controls. Cell culture experiments revealed that patient-derived XMRV is infectious and that both cell-associated and cell-free transmission of the virus are possible. Secondary viral infections were established in uninfected primary lymphocytes and indicator cell lines after their exposure to activated PBMCs, B cells, T cells, or plasma derived from CFS patients. These findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS.

Chronic fatigue syndrome (CFS) is a disorder of unknown etiology that affects multiple organ systems in the body. Patients with CFS display abnormalities in immune sys-

tem function, often including chronic activation of the innate immune system and a deficiency in natural killer cell activity (1, 2). A number of viruses, including ubiquitous herpesviruses and

enteroviruses, have been implicated as possible environmental triggers of CFS (1). Patients with CFS often have active β herpesvirus infections, suggesting an underlying immune deficiency.

The recent discovery of a gammaretrovirus, xenotropic murine leukemia virus-related virus (XMRV), in the tumor tissue of a subset of prostate cancer patients prompted us to test whether XMRV might be associated with CFS. Both of these disorders, XMRV-positive prostate cancer and CFS, have been linked to alterations in the antiviral enzyme RNase L (3–5). Using the Whittemore Peterson Institute's (WPI's) national

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tissue repository, which contains samples from well-characterized cohorts of CFS patients, we isolated nucleic acids from PBMCs and assayed the samples for XMRV *gag* sequences by nested polymerase chain reaction (PCR) (5, 6). Of the 101 CFS samples analyzed, 68 (67%) contained XMRV *gag* sequence. Detection of XMRV was confirmed in 7 of 11 WPI CFS samples at the Cleveland Clinic by PCR-amplifying and sequencing segments of XMRV *env* [352 nucleotides (nt)] and *gag* (736 nt) in CFS PBMC DNA (Fig. 1A) (6). In contrast, XMRV *gag* sequences were detected in 8 of 218 (3.7%) PBMC DNA specimens from healthy individuals. Of the 11 healthy control DNA samples analyzed by PCR for both *env* and *gag*, only one sample was positive for *gag* and none for *env* (Fig. 1B). In all positive cases, the XMRV *gag* and *env* sequences were more than 99% similar to those previously reported for prostate tumor-associated strains of XMRV (VP62, VP35, and VP42) (fig. S1) (5).

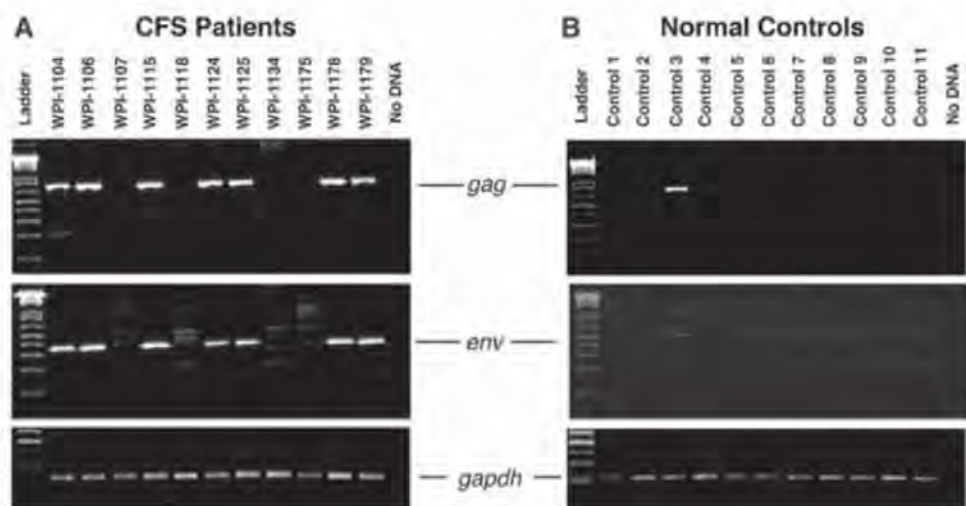


Fig. 1. XMRV sequences in PBMC DNA from CFS patients. Single-round PCR results for *gag*, *env*, and *gapdh* sequences in PBMCs of (A) CFS patients and (B) healthy controls are shown. The positions of the amplicons are indicated and DNA markers (ladder) are shown. These are representative results from one group of 20 patients.

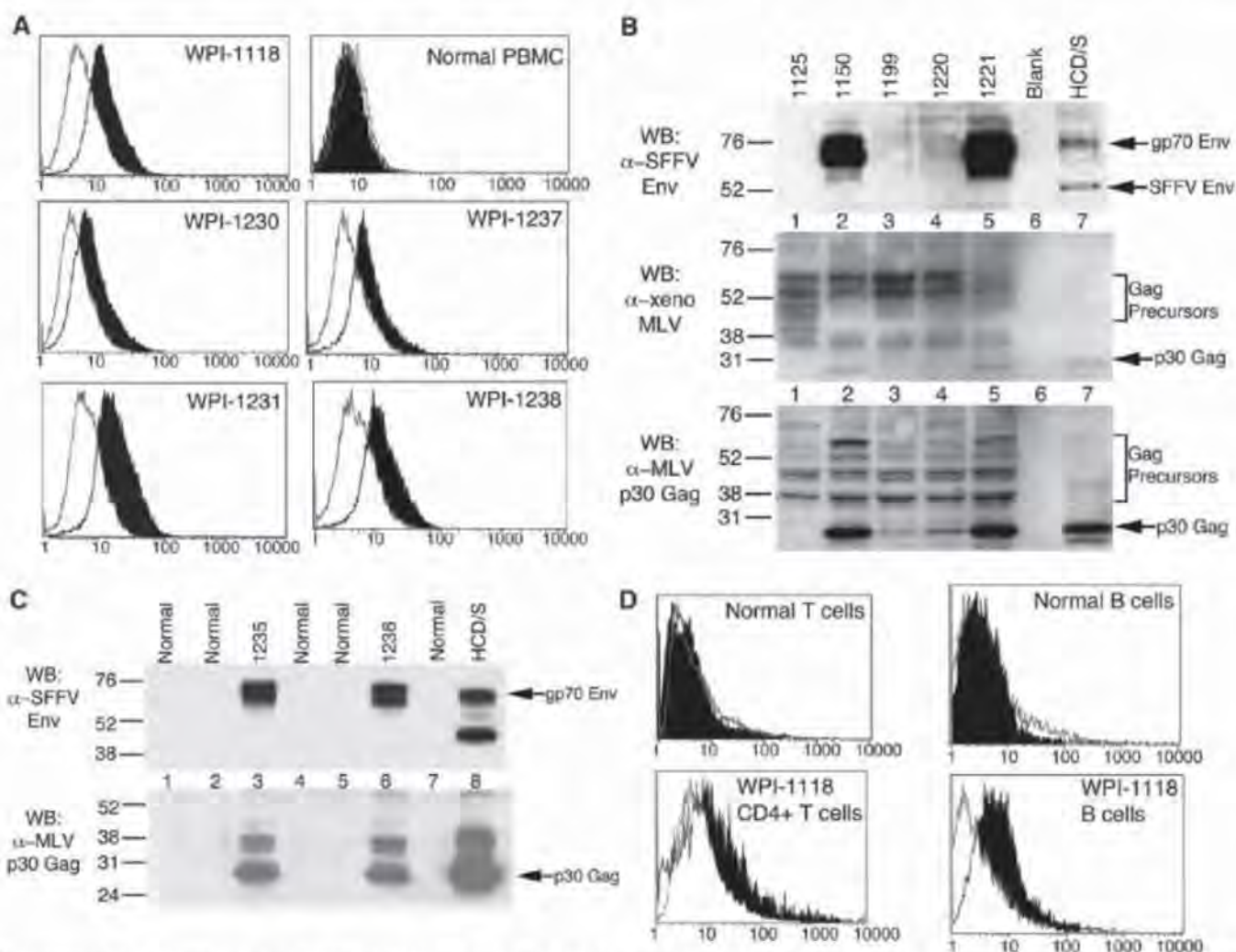


Fig. 2. Expression of XMRV proteins in PBMCs from CFS patients. (A) PBMCs were activated with phytohemagglutinin and interleukin-2, reacted with a mAb to MLV p30 Gag, and analyzed by IFC. (B) Lysates of activated PBMCs from CFS patients (lanes 1 to 5) were analyzed by Western blots with rat mAb to SFFV Env (top panel), goat antiserum to xenotropic MLV (middle panel), or goat antiserum to MLV p30 Gag (bottom panel). Lane 7, lysate from SFFV-infected HCD-57 cells. Molecular weight markers in kilodaltons are at left. (C)

Lysates of activated PBMCs from healthy donors (lanes 1, 2, 4, 5, and 7) or from CFS patients (lanes 3 and 6) were analyzed by Western blots using rat mAb to SFFV Env (top panel) or goat antiserum to MLV p30 Gag (bottom panel). Lane 8, SFFV-infected HCD-57 cells. Molecular weight (MW) markers in kilodaltons are at left. (D) CD4⁺ T cells (left) or CD19⁺ B cells (right) were purified, activated, and examined by flow cytometry for XMRV Gag with a mAb to MLV p30 Gag.

Sequences of full-length XMRV genomes from two CFS patients and a partial genome from a third patient were generated (table S1). CFS XMRV strains 1106 and 1178 each differed by 6 nt from

the reference prostate cancer strain XMRV VP62 (EF185282), and with the exception of 1 nt, the variant nucleotides mapped to different locations within the XMRV genome, suggesting indepen-

dent infections. In comparison, prostate cancer-derived XMRV strains VP35 and VP42 differed from VP62 by 13 and 10 nt, respectively. Thus, the complete XMRV genomes in these CFS patients

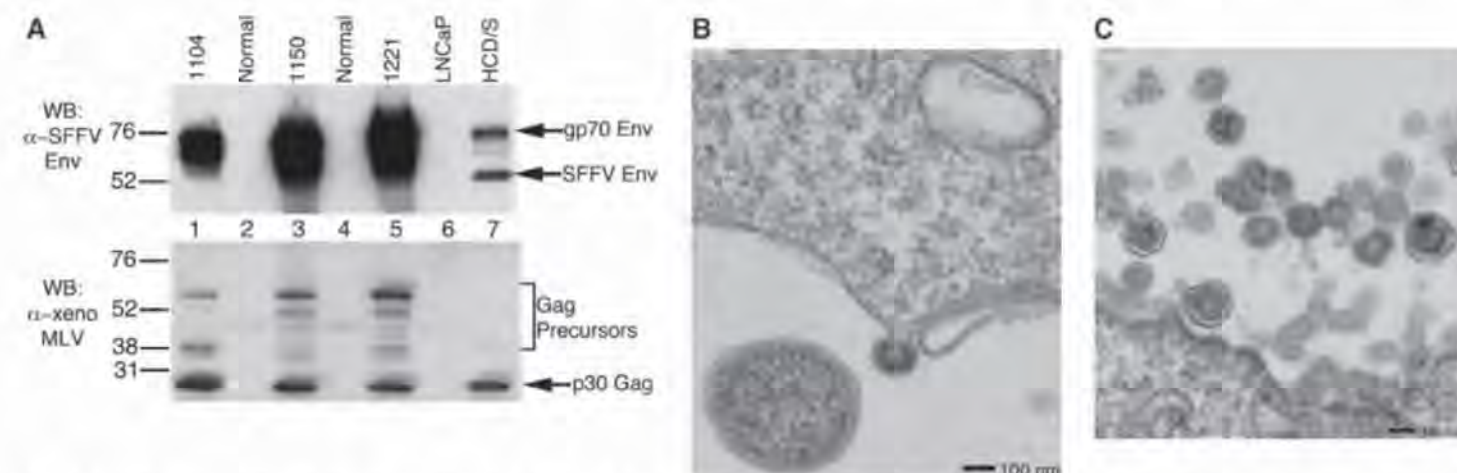


Fig. 3. Infectious XMRV in PBMCs from CFS patients. (A) Lysates of LNCaP cells cocultured with PBMCs from CFS patients (lanes 1, 3, and 5) or healthy donors (lanes 2 and 4) were analyzed by Western blots with rat mAb to SFFV Env (top panel) or goat antiserum to xenotropic MLV (bottom panel). Lane 6, uninfected LNCaP; lane 7,

SFFV-infected HCD-57 cells. MW markers in kilodaltons are at left. (B) Transmission electron micrograph of a budding viral particle from LNCaP cells infected by incubation with an activated T cell culture from a CFS patient. (C) Transmission electron micrograph of virus particles released by infected LNCaP cells.

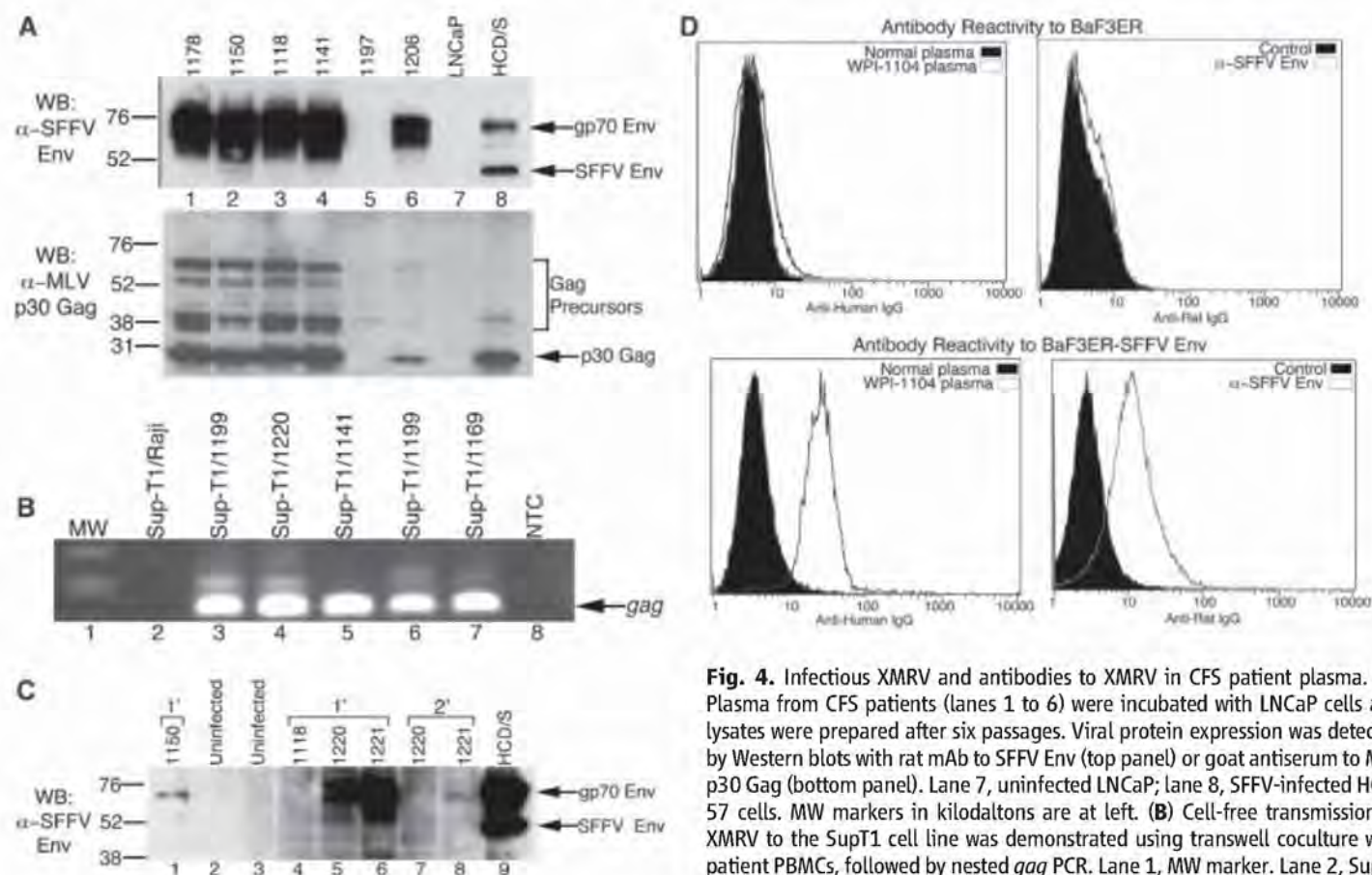


Fig. 4. Infectious XMRV and antibodies to XMRV in CFS patient plasma. (A) Plasma from CFS patients (lanes 1 to 6) were incubated with LNCaP cells and lysates were prepared after six passages. Viral protein expression was detected by Western blots with rat mAb to SFFV Env (top panel) or goat antiserum to MLV p30 Gag (bottom panel). Lane 7, uninfected LNCaP; lane 8, SFFV-infected HCD-57 cells. MW markers in kilodaltons are at left. (B) Cell-free transmission of XMRV to the SupT1 cell line was demonstrated using transwell coculture with patient PBMCs, followed by nested *gag* PCR. Lane 1, MW marker. Lane 2, SupT1 cocultured with Raji. Lanes 3 to 7, SupT1 cocultured with CFS patient PBMCs. Lane 8, no template control (NTC). (C) Normal T cells were exposed to cell-free supernatants obtained from T cells (lanes 1, 5, and 6) or B cells (lane 4) from CFS patients. Lanes 7 and 8 are secondary infections of normal activated T cells. Initially, uninfected primary T cells were exposed to supernatants from PBMCs of patients WPI-1220 (lane 7) and WPI-1221 (lane 8). Lanes 2 and 3, uninfected T cells; lane 9, SFFV-infected HCD-57 cells. Viral protein expression was detected by Western blot with a rat mAb to SFFV Env. MW markers in kilodaltons are at left. (D) Plasma samples from a CFS patient or from a healthy control as well as SFFV Env mAb or control were reacted with BaF3ER cells (top) or BaF3ER cells expressing recombinant SFFV Env (bottom) and analyzed by flow cytometry. IgG, immunoglobulin G.

were >99% identical in sequence to those detected in patients with prostate cancer. To exclude the possibility that we were detecting a murine leukemia virus (MLV) laboratory contaminant, we determined the phylogenetic relationship among endogenous (non-ecotropic) MLV sequences, XMRV sequences, and sequences from CFS patients 1104, 1106, and 1178 (fig. S2). XMRV sequences from the CFS patients clustered with the XMRV sequences from prostate cancer cases and formed a branch distinct from non-ecotropic MLVs common in inbred mouse strains. Thus, the virus detected in the CFS patients' blood samples is unlikely to be a contaminant.

To determine whether XMRV proteins were expressed in PBMCs from CFS patients, we developed intracellular flow cytometry (IFC) and Western blot assays, using antibodies (Abs) with novel viral specificities. These antibodies included, among others, (i) rat monoclonal antibody (mAb) to the spleen focus-forming virus (SFFV) envelope (Env), which reacts with all polytropic and xenotropic MLVs (7); (ii) goat antisera to whole mouse NZB xenotropic MLV; and (iii) a rat mAb to MLV p30 Gag (8). All of these Abs detected the human VP62 XMRV strain grown in human Raji, LNCaP, and Sup-T1 cells (fig. S3) (5). IFC of activated lymphocytes (6, 9) revealed that 19 of 30 PBMC samples from CFS patients reacted with the mAb to MLV p30 Gag (Fig. 2A). The majority of the 19 positive samples also reacted with antisera to other purified MLV proteins (fig. S4A). In contrast, 16 healthy control PBMC cultures tested negative (Fig. 2A and fig. S4A). These results were confirmed by Western blots (Fig. 2, B and C) (6) using Abs to SFFV Env, mouse xenotropic MLV, and MLV p30 Gag. Samples from five healthy donors exhibited no expression of XMRV proteins (Fig. 2C). The frequencies of CFS cases versus healthy controls that were positive and negative for XMRV sequences were used to calculate a Pearson χ^2 value of 154 (two-tailed *P* value of 8.1×10^{-35}). These data yield an odds ratio of 54.1 (a 95% confidence interval of 23.8 to 122), suggesting a nonrandom association with XMRV and CFS patients.

To determine which types of lymphocytes in blood express XMRV, we isolated B and T cells from one patient's PBMCs (6). Using mAb to MLV p30 Gag and IFC, we found that both activated T and B cells were infected with XMRV (Fig. 2D and fig. S4A). Furthermore, using mAb to SFFV Env, we found that >95% of the cells in a B cell line developed from another patient were positive for XMRV Env (fig. S4B). XMRV protein expression in CFS patient-derived activated T and B cells grown for 42 days in culture was confirmed by Western blots (fig. S4C) using Abs to SFFV Env and xenotropic MLV.

We next investigated whether the viral proteins detected in PBMCs from CFS patients represent infectious XMRV. Activated lymphocytes (6) were cocultured with LNCaP, a prostate cancer cell line with defects in both the JAK-STAT and RNase L pathways (10, 11) that was previ-

ously shown to be permissive for XMRV infection (12). After coculture with activated PBMCs from CFS patients, LNCaP cells expressed XMRV Env and multiple XMRV Gag proteins when analyzed by Western blot (Fig. 3A) and IFC (fig. S5A). Transmission electron microscopy (EM) of the infected LNCaP cells (Fig. 3B), as well as virus preparations from these cells (Fig. 3C), revealed 90- to 100-nm-diameter budding particles consistent with a gamma (type C) retrovirus (13).

We also found that XMRV could be transmitted from CFS patient plasma to LNCaP cells when we applied a virus centrifugation protocol to enhance infectivity (6, 14, 15). Both XMRV gp70 Env and p30 Gag were abundantly expressed in LNCaP cells incubated with plasma samples from 10 of 12 CFS patients, whereas no viral protein expression was detected in LNCaP cells incubated with plasma samples from 12 healthy donors (Fig. 4A). Likewise, LNCaP cells incubated with patient plasma tested positive for XMRV p30 Gag in IFC assays (fig. S5B). We also observed cell-free transmission of XMRV from the PBMCs of CFS patients to the T cell line SupT1 (Fig. 4B) and both primary and secondary transmission of cell-free virus from the activated T cells of CFS patients to normal T cell cultures (Fig. 4C). Together, these results suggest that both cell-associated and cell-free transmission of CFS-associated XMRV are possible.

We next investigated whether XMRV stimulates an immune response in CFS patients. For this purpose, we developed a flow cytometry assay that allowed us to detect Abs to XMRV Env by exploiting its close homology to SFFV Env (16). Plasma from 9 out of 18 CFS patients infected with XMRV reacted with a mouse B cell line expressing recombinant SFFV Env (BaF3ER-SFFV-Env) but not to SFFV Env negative control cells (BaF3ER), analogous to the binding of the SFFV Env mAb to these cells (Fig. 4D and S6A). In contrast, plasma from seven healthy donors did not react (Fig. 4D and fig. S6A). Furthermore, all nine positive plasma samples from CFS patients but none of the plasma samples from healthy donors blocked the binding of the SFFV Env mAb to SFFV Env on the cell surface (fig. S6B). These results are consistent with the hypothesis that CFS patients mount a specific immune response to XMRV.

Neurological maladies and immune dysfunction with inflammatory cytokine and chemokine up-regulation are some of the most commonly reported features associated with CFS. Several retroviruses, including the MLVs and the primate retroviruses HIV and HTLV-1, are associated with neurological diseases as well as cancer (17). Studies of retrovirus-induced neurodegeneration in rodent models have indicated that vascular and inflammatory changes mediated by cytokines and chemokines precede the neurological pathology (18, 19). The presence of infectious XMRV in lymphocytes may account for some of these observations of altered immune responsiveness and neurological function in CFS patients.

We have discovered a highly significant association between the XMRV retrovirus and CFS. This observation raises several important questions. Is XMRV infection a causal factor in the pathogenesis of CFS or a passenger virus in the immunosuppressed CFS patient population? What is the relationship between XMRV infection status and the presence or absence of other viruses that are often associated with CFS (e.g., herpesviruses)? Conceivably these viruses could be cofactors in pathogenesis, as is the case for HIV-mediated disease, in which co-infecting pathogens play an important role (20). Patients with CFS have an elevated incidence of cancer (21). Does XMRV infection alter the risk of cancer development in CFS? As noted above, XMRV has been detected in prostate tumors from patients expressing a specific genetic variant of the *RNASEL* gene (5). In contrast, in our study of this CFS cohort, we found that XMRV infection status does not correlate with the *RNASEL* genotype (6) (table S2).

Finally, it is worth noting that 3.7% of the healthy donors in our study tested positive for XMRV sequences. This suggests that several million Americans may be infected with a retrovirus of as yet unknown pathogenic potential.

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Note added in proof: V.C.L. is operations manager of Viral Immune Pathologies Laboratory, which is in negotiations

with the Whittemore Peterson Institute to offer a diagnostic test for XMRV.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1179052/DC1

Materials and Methods

Figs. S1 to S6

Tables S1 and S2
References

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Complete Reconstitution of a Highly Reducing Iterative Polyketide Synthase

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Highly reducing iterative polyketide synthases are large, multifunctional enzymes that make important metabolites in fungi, such as lovastatin, a cholesterol-lowering drug from *Aspergillus terreus*. We report efficient expression of the lovastatin nonaketide synthase (LovB) from an engineered strain of *Saccharomyces cerevisiae*, as well as complete reconstitution of its catalytic function in the presence and absence of cofactors (the reduced form of nicotinamide adenine dinucleotide phosphate and S-adenosylmethionine) and its partner enzyme, the enoyl reductase LovC. Our results demonstrate that LovB retains correct intermediates until completion of synthesis of dihydromonacolin L, but off-loads incorrectly processed compounds as pyrones or hydrolytic products. Experiments replacing LovC with analogous MlcG from compactin biosynthesis demonstrate a gate-keeping function for this partner enzyme. This study represents a key step in the understanding of the functions and structures of this family of enzymes.

Nature uses an amazing array of enzymes to make natural products (1). Among these metabolites, polyketides represent a class of over 7000 known structures of which more than 20 are commercial drugs (2). Among the most interesting but least understood enzymes making these compounds are the highly reducing iterative polyketide synthases (HR-IPKs) found in filamentous fungi (3). In contrast to the well-studied bacterial type I PKs that operate in an assembly line fashion (4), HR-IPKs are megasynthases that function iteratively by using a set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites (5). One such metabolite is lovastatin, a cholesterol-lowering drug from *Aspergillus terreus* (6). This compound is a precursor to simvastatin (Zocor, Merck, Whitehouse Station, NJ), a semi-synthetic drug that had annual sales of more than \$4 billion before loss of patent protection in 2006 (7).

Biosynthesis of lovastatin proceeds via dihydromonacolin L (acid form 1, lactone form 2), a product made by the HR-IPKs lovastatin nonaketide synthase (LovB), with the assistance of a separate enoyl reductase, LovC (8) (Fig. 1). LovB is a 335-kD protein that contains single copies of

ketosynthase (KS), malonyl-coenzyme A (CoA) acyltransferase (MAT), dehydratase (DH), methyltransferase (MT), ketoreductase (KR), and acyl-carrier protein (ACP) domains, as well as a section that is homologous to the condensation (CON) domain found in nonribosomal peptide synthetases (NRPSs) (9). It also contains a domain that resembles an enoyl reductase (ER) but lacks that activity. LovB must catalyze ~35 reactions and use different permutations of tailoring domains after each of the eight chain-extension steps to yield the nonaketide, dihydromonacolin L (2). This enzyme also catalyzes a biological Diels-Alder reaction during the assembly process to form the decalin ring system (10). In vitro studies of LovB (11) have been hampered by an inability to obtain sufficient amounts of the functional purified megasynthase from either *A. terreus* or heterologous *Aspergillus* hosts. As a result, the programming that governs metabolite assembly by LovB or other HR-IPKs is not understood. Key aspects that remain to be elucidated include (i) the catalytic and structural roles of each domain in the megasynthase, (ii) substrate specificities of the catalytic domains and their tolerance to perturbation in megasynthase functions, and (iii) factors governing the choice of different combinations of domains during each iteration of catalysis. To initiate such studies, we engineered an expression system in yeast to produce large amounts of LovB and examined the influence of cofactors and the ER partner (e.g., LovC) on product formation.

The engineered *Saccharomyces cerevisiae* strain BJ5464-NpgA, which contains a chromo-

somal copy of the *Aspergillus nidulans* phosphopantetheinyl (ppant) transferase gene *npgA* (12), was the expression host. A C-terminal hexahistidine-tagged LovB was placed under the control of the *S. cerevisiae ADHI2* promoter (13, 14) on an episomal plasmid (YEplLovB-6His). Abundant amounts of the intact LovB could be purified from the soluble fraction to near homogeneity with a final yield of ~4.5 mg/L (fig. S1). We used mass analysis of tryptic digest fragments to verify the identity of the recombinant LovB. The ACP domain of LovB was determined to be nearly completely phosphopantetheinylated by using a ppant ejection assay with high-resolution quadrupole orthogonal acceleration-time-of-flight mass spectrometry (fig. S2). To ascertain activity of the resulting LovB and to examine the necessity for cofactors, malonyl-CoA alone was first added to the purified enzyme in buffer. Whole-cell feeding studies of doubly [¹³C, ³H]-labeled acetate to cultures of *A. terreus* showed that all three acetate hydrogens were incorporated into the acetate-derived starter units for both the nonaketide and diketide moieties in lovastatin (15). The purified LovB can use malonyl-CoA for both chain priming and chain elongation, loading malonate with decarboxylation to make the acetyl starter unit. Although LovB is able to prime with and elongate the chain by two further condensations with malonyl-CoA, in the absence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), no ketoreduction occurs. The dominant product is lactone 3 (Fig. 2A, trace i), which forms by enolization and cyclization with off-loading of the unreduced triketide. Addition of NADPH to this system enables function of the KR domain. In this and subsequent experiments, the malonyl-CoA could be conveniently synthesized in situ by malonyl-CoA synthase (MatB) from *Rhizobium trifolii* using free malonate and CoA (16). With KR enabled, LovB makes penta-, hexa-, and heptaketide pyrones 4 to 6, as well as ketones 7 and 8 (Fig. 2A, trace ii). The structures were confirmed by chemical synthesis of authentic standards, except for heptaketide 6, which proved very unstable. However, the mass increase of 26 atomic mass units for 6 and its red shift in the ultraviolet spectrum when compared to 5 are consistent with its proposed heptaketide pyrone structure (table S3). Compounds 7 and 8 result from thioester hydrolysis of penta- and hexaketides stalling on the ACP at the β-keto stage. The resulting β-keto acids spontaneously decarboxylate to afford 7 and 8. Formation of compounds 4 to 8 illustrates that derailment in the normal programmed steps, namely the lack of methylation due to the absence of S-adenosylmethionine

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(SAM), stalls chain elongation and promotes off-loading from the ACP. This occurs either by the addition of two acetate units without reduction, followed by pyrone formation (4 to 6, major route) or by the addition of one acetate unit and hydrolysis/decarboxylation of the shunt intermediates (7 and 8, minor route) (Fig. 2B). In either case, the shut-down of chain elongation and re-

ductive processing is not absolute: LovB is able to create longer homologs (i.e., 5, 6, and 8) after failure of methylation at the tetraketide stage.

SAM was then added to the system to enable construction of the natural intermediate, methylated tetraketide 11. However, as enoyl reduction cannot occur in the absence of LovC, methylated hexa- and heptaketide pyrones 9 and 10 are the

primary products (Fig. 2A, trace iii). This is consistent with previous results where 9 and 10 are generated when LovB is expressed in *A. nidulans* in the absence of LovC, or when LovC is inactivated in the producer *A. terreus* (8).

Methylated tetraketide 11 must undergo enoyl reduction en route to 2. To examine the influence of the ER, active LovC was added to LovB,

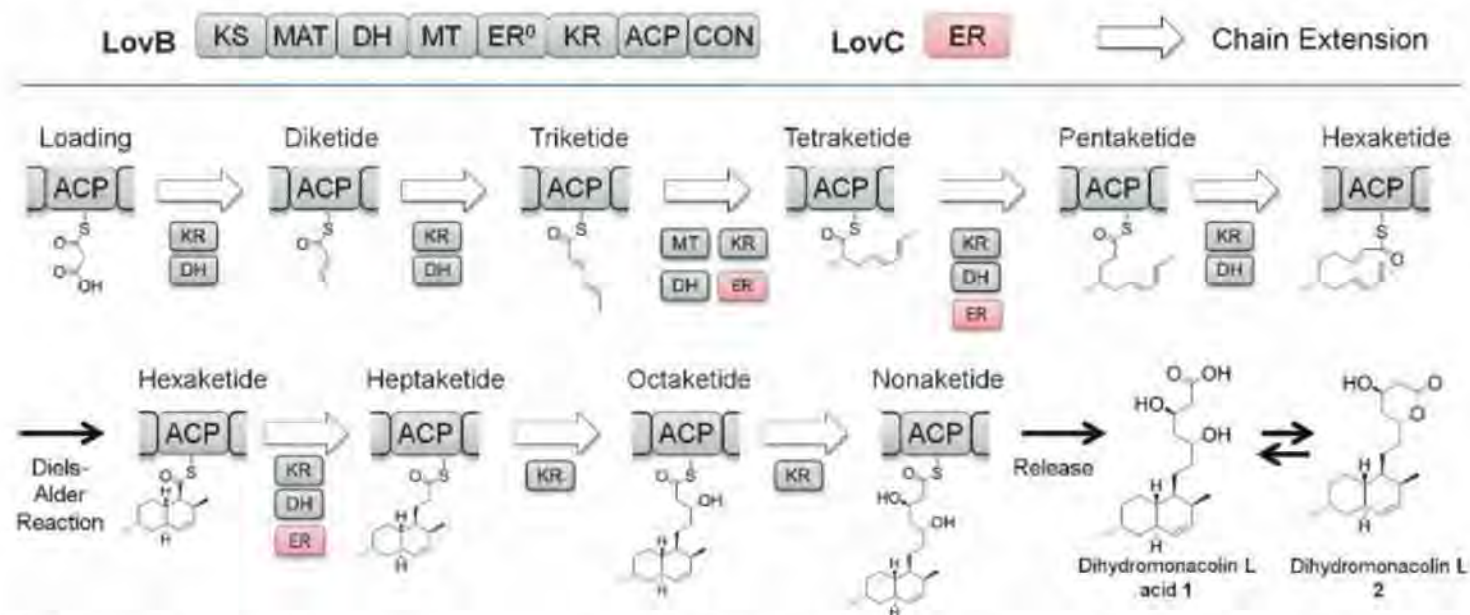
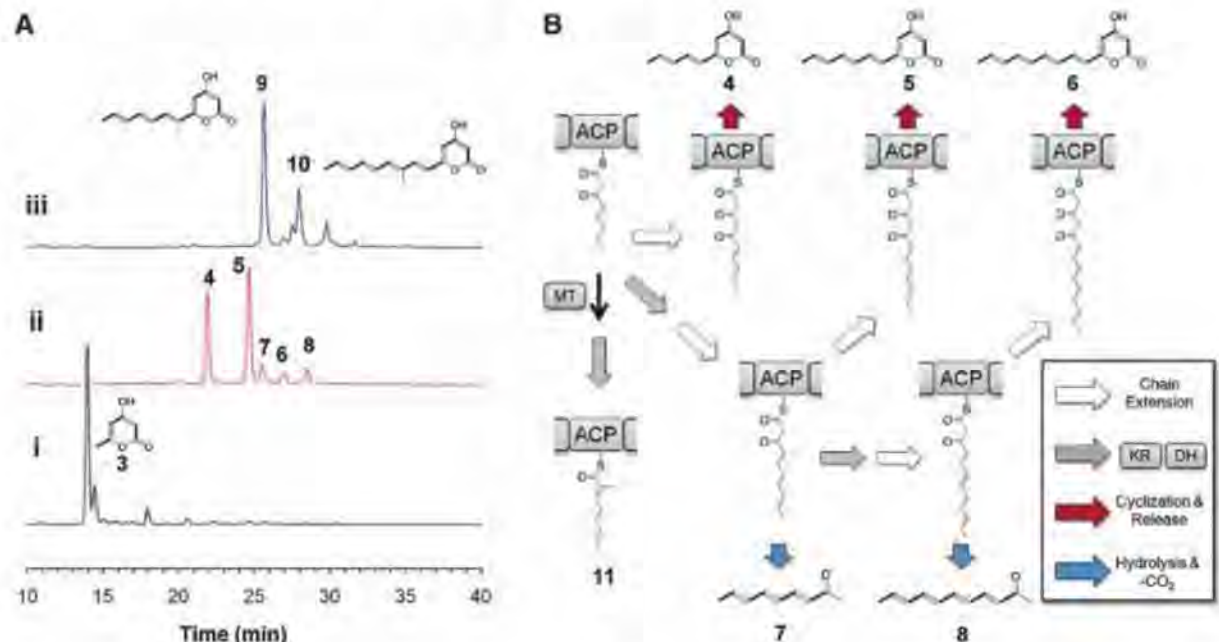


Fig. 1. Proposed mechanism of dihydromonacolin L 1 synthesis by LovB and the accessory ER LovC. LovB (335 kD) consists of eight discrete domains and operates iteratively to condense nine malonyl-CoA equivalents to yield the nonaketide product 1. Loading of the megasynthase by malonyl-CoA is presumably followed by decarboxylation to yield the acetyl starter unit (not shown). Each round of Claisen condensation is catalyzed by the KS domain, whereas the growing polyketide is tethered to the phosphopantetheinyl (shown with a squiggle line) arm of the ACP. After each condensation, the polyketide is

subjected to a different combination of tailoring, which can include α -methylation by the MT domain, β -ketoreduction by the KR domain, β -dehydration by the DH domain, and α - β -enoylreduction by the dissociated LovC. The different tailoring permutations after each round of chain extension yield a triene-containing hexaketide thioester that can undergo a stereospecific Diels-Alder cyclization to yield the decalin portion of 1. After formation of the nonaketide, the chain is released to yield the ring-open form 1. The acid form of 1 can undergo lactonization to yield 2.

Fig. 2. Polyketides synthesized by LovB under different assay conditions in the absence of LovC. (A) (Trace i) Reaction with LovB afforded the triketide lactone 3, which confirms the function of the minimal PKS domains of LovB. (Trace ii) Reaction with LovB and NADPH (2 mM) afforded a number of highly conjugated compounds (4 to 8). (Trace iii) Reaction with LovB, NADPH (2 mM), and SAM (2 mM) afford the methylated, conjugated pyrones 9 and 10. All reactions used MatB to regenerate malonyl-CoA and were extracted with ethyl acetate (EA)/acetic acid (AcOH) (ratio of 99/1);



(B) Fate of the tetraketide intermediate in the absence of SAM and LovC. The correct tailoring yields the key intermediate 11. In the absence of α -methylation, pyrones 4 to 6 can form readily through cyclization and release, whereas the ketones 7 and 8 can form via hydrolysis and decarboxylation.

NADPH, and malonyl-CoA in the absence of SAM. It might be expected that a des-methyl version of dihydromonacolin L (i.e., **12**) would be produced, but compounds **5** to **8** are obtained instead. This suggests that the α -methyl substitution, although not required for the function of KR and DH, is a prerequisite for enoyl reduction of the tetraketide. One possibility is that LovC has stringent substrate specificity at the tetraketide stage for an α -methyl substituted chain. Alternatively, absence of SAM or of a methylated substrate chain could prevent LovB from interacting with LovC.

We then attempted to reconstitute the synthesis of dihydromonacolin L **1** in vitro. Equimolar amounts (25 μ M) of purified LovB and LovC were incubated at 25°C for 12 hours with all cofactors (NADPH, SAM) and malonyl-CoA. The resulting sample was extracted under acidic conditions to convert any **1** into **2**. However, liquid chromatography-mass spectrometry (LC-MS) analysis revealed that **2** was not present in the extract (Fig. 3A, trace ii). No truncated products are observed, which suggests that LovB may not be able to release **1** because it lacks a thioesterase (TE) domain to free the completed product. To examine if the polyketide remains covalently attached to the ACP domain of LovB, we performed an analogous experiment in which the mixture was treated with base to hydrolyze thioester bonds

before acidic extraction. LC-MS analysis revealed the emergence of a mass/charge ratio (m/z) $[M+H]^+$ 307 ion eluting at the exact retention time of the standard **2** (Fig. 3A, trace iii, and fig. S11). As expected, repetition of the experiment with $[2-^{13}C]$ malonate gives a peak at the same retention time with m/z $[M+H]^+$ 316 (Fig. 3A, trace iv, and table S3). Time-course analysis reveals that ~30 ng of **2** can be recovered at the plateau level (fig. S12), which corresponds to ~3% of the theoretical yield of the single-turnover experiment and suggests that some of the LovB is inactive. The reconstituted system possesses the entire range of catalytic activities and does not generate truncated products.

The failure of LovB to release **1** is surprising because the heterologous host *A. nidulans* expressing LovB and LovC produces substantial quantities of dihydromonacolin L (**8**). Cleavage of the LovB ACP-thioester of **1** may be achieved by TEs involved in fatty acid biosynthesis or unrelated PKS pathways. To test this idea, an excised fungal TE domain from the *Gibberella zeae* PKS13 (**17**), which has broad specificity, was added to the in vitro reaction. This action leads to release of **1** and enzyme turnover, which can be detected by production of lactone **2** after mild acid extraction (no base added) (Fig. 3A, trace v). Time-course analysis (fig. S12) demonstrates that PKS13 TE can support the generation of **1** lin-

early for ~12 hours to give a >10-fold increase in product. Turnover is dependent on the catalytic triad of PKS13 TE, as mutation of its active site His²⁰⁰⁹ to Ala²⁰⁰⁹ completely abolishes the release of **1** (fig. S12). Other fungal TE domains, such as the *Gibberella fujikuroi* PKS4 TE domain (**18**), also allow liberation of **1**, albeit at 50% of the rate of PKS13 TE. In contrast, TE domains from bacterial type I PKSs, such as that from the erythromycin PKS (**19**), do not show any detectable release of **1** (fig. S12). These experiments suggest that cleavage of **1** from LovB in *Aspergillus* can proceed via TEs not present in the gene cluster for lovastatin biosynthesis.

We next examined whether the CON domain in LovB is required for synthesis of **1**. This portion of LovB could be an evolutionary relic derived from a fungal PKS-NRPS hybrid in which an entire NRPS module (consisting of CON, adenylation, and peptidyl carrier protein domains) is present (**20**, **21**). With the use of *S. cerevisiae* BJ5464-NpgA, a soluble truncated variant LovB- Δ C was expressed that terminates at the end of the ACP boundary (Ser²⁵⁴²) and lacks the CON domain. A combination of purified LovB- Δ C (in place of full-length LovB) and LovC, PKS13 TE, and the other required cofactors does not produce detectable amounts of **2** (fig. S14). A single-turnover experiment (using base hydro-

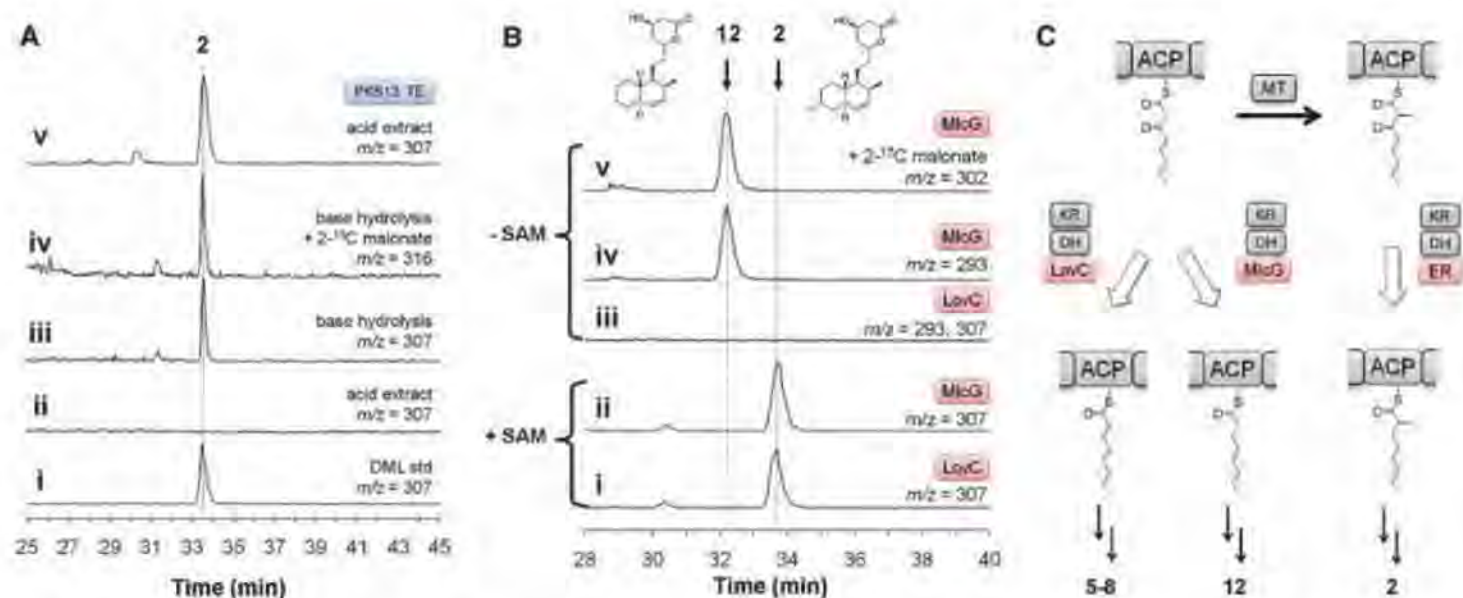


Fig. 3. Synthesis of **2** and **12** by LovB and dissociated ER in vitro. (A) LC-MS analysis of the synthesis of **2** using purified LovB and LovC. Typical reaction contains 25 μ M of LovB and LovC, 2 mM NADPH, 2 mM SAM, and the MatB malonyl-CoA regeneration components (see supporting online material for details). The traces shown are the selected ion monitoring of desired ions in the positive ionization mode. (Trace i) Standard of **2** purified from *A. nidulans* (**8**). DML, dihydromonacolin L. (Trace ii) Products recovered from the reaction after extraction with EA/AcOH (99/1 ratio). (Trace iii) The reaction mixture was first treated with 1 M KOH at 65°C for 10 min to hydrolyze all attached polyketides, followed by acidification with 1 N HCl to pH 2.0, followed by extraction. Emergence of **2** indicates the products are not released by LovB under reaction conditions. (Trace iv) Products recovered from base-treated sample that used $[2-^{13}C]$ -malonate as the precursor. The +9 mass shift confirms the nonaketide backbone of **2** synthesized by LovB. (Trace v) Release of **2** from LovB by

heterologous fungal *G. zeae* PKS13 TE (25 μ M). No base hydrolysis is required for observed product release. (B) Synthesis of **12** in the absence of SAM requires a different ER (MlcG) from the compactin biosynthetic pathway. All reactions contain LovB, PKS13 TE, ER, and 2 mM NADPH and were extracted with EA/TFA (99/1 ratio). Products were detected with the use of selected positive ion monitoring of 307 for **2**, 293 for **12**, and 302 for **12** synthesized from $[2-^{13}C]$ -malonate. (Traces i and ii) Both LovC and MlcG can work with LovB to produce **2** in the presence of 2 mM SAM. (Trace iii) In the absence of SAM, LovC cannot reduce the tetraketide intermediate, and shunt products **5** to **8** were synthesized (not shown). (Trace iv) In the absence of SAM, MlcG can perform the enoyl reduction and lead to the synthesis of **12**. (Trace v) When $[2-^{13}C]$ -malonate was used, the expected +9 mass increase was observed in **12** using LovB and MlcG. (C) The difference in substrate specificity between LovC and MlcG toward methylated and unmethylated tetraketide intermediate.

ysis workup) with LovB- Δ C, LovC, and required cofactors (but no PKS13 TE) did not yield compound **2** (fig. S14). Removal of the CON domain does not affect the functions of the minimal PKS and the tailoring domains. The synthesis of compounds **3**, **4** to **8**, or **9** and **10** proceeds as efficiently as it does with full-length LovB using LovB- Δ C alone, LovB- Δ C with NADPH, or LovB- Δ C with NADPH and SAM, respectively. The stand-alone CON protein (**22**) was then added in equimolar amount to the LovB- Δ C. Remarkably, the CON domain can interact with LovB- Δ C in trans and afford **2** in both the single-turnover (base hydrolysis workup) and the PKS13 TE-mediated release assays (fig. S14). The yields of **2** in both assays are lower than with the full-length LovB, which may be due to the less efficient in trans protein-protein interactions. These in vitro experiments indicate a critical but still enigmatic role for this domain in controlling correct biosynthesis of **1**.

To examine whether replacement of the in trans interaction of LovB with LovC is possible, we cloned MlcG, an analogous, dissociated ER from the compactin biosynthetic gene cluster in *Penicillium citrinum* (fig. S3) (**23**). Compactin is a 6-desmethyl analog of lovastatin, and **12** is a proposed intermediate in its biosynthesis. Hence, the compactin nonaketide synthase MlcA and its ER partner MlcG are programmed to function normally in the absence of methylation at the tetraketide stage, in contrast to the LovB/LovC complex. Substitution of LovC with MlcG (72% identity, 83% similarity to LovC) in the absence of SAM affords desmethyl-dihydromonacolin L **12** in vitro instead of pyrones **5** to **8** (Fig. 3B, trace iv). Chemical synthesis of optically pure **12** as a standard confirmed its identity. The yield and the turnover rate (with PKS13 TE) for **12** are

similar to those for **2**, indicating that skipping the methylation step has little effect on later stages of LovB synthesis. Addition of SAM to LovB and MlcG affords **2** in similar yield as the native enzyme pairing of LovB and LovC (Fig. 3B, traces i and ii), thereby demonstrating that MlcG is tolerant of both methylated and unmethylated tetraketide substrates (Fig. 3C). Thus, LovC is highly specific toward a methylated tetraketide, which is surprising considering that it must be capable of reducing α -unmethylated pentaketide and heptaketide intermediates during the biosynthesis of **1**. It is clear from the ability of MlcG and LovB to produce **12** in the absence of SAM (or **2** when this cofactor is added) that the gate-keeping mechanism for the normal synthesis in *A. terreus* resides with the ER LovC.

We demonstrated characterization of a purified HR-IPKS system by reconstituting in vitro the entire range of activities of LovB and its partner LovC. Together, these two enzymes catalyze the synthesis of **1** with excellent control of processivity, stereochemistry, and regioselectivity. Addition of heterologous TE domains facilitates the release of the final product. This approach opens the door to structural analysis of the proteins with partly assembled intermediates and provides a basis for understanding the programming rules of HR-IPKSs.

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Supporting Online Material

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SOM Text

Figs. S1 to S16

Tables S1 to S4

References

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PTP σ Is a Receptor for Chondroitin Sulfate Proteoglycan, an Inhibitor of Neural Regeneration

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Chondroitin sulfate proteoglycans (CSPGs) present a barrier to axon regeneration. However, no specific receptor for the inhibitory effect of CSPGs has been identified. We showed that a transmembrane protein tyrosine phosphatase, PTP σ , binds with high affinity to neural CSPGs. Binding involves the chondroitin sulfate chains and a specific site on the first immunoglobulin-like domain of PTP σ . In culture, PTP σ ^{-/-} neurons show reduced inhibition by CSPG. A PTP σ fusion protein probe can detect cognate ligands that are up-regulated specifically at neural lesion sites. After spinal cord injury, PTP σ gene disruption enhanced the ability of axons to penetrate regions containing CSPG. These results indicate that PTP σ can act as a receptor for CSPGs and may provide new therapeutic approaches to neural regeneration.

Recovery after central nervous system (CNS) injury is minimal, leading to substantial current interest in potential strategies to overcome this challenge (1–5). Chondroitin sul-

fate proteoglycans (CSPGs) show dramatic up-regulation after neural injury, within the extracellular matrix of scar tissue and in the perineuronal net within more-distant targets of the severed axons

(6, 7). The inhibitory nature of CSPGs is reflected not only in the formation of dystrophic axonal retraction bulbs that fail to regenerate through the lesion (8), but also in the limited capacity for collateral sprouting of spared fibers (8, 9). This inhibition can be relieved by chondroitinase ABC digestion of the chondroitin sulfate (CS) side chains, which can promote regeneration and sprouting and restore lost function (10–14). It has been known for nearly two decades that sulfated proteoglycans are major contributors to the repulsive nature of the glial scar (15); however, the precise inhibitory mechanism remains poorly understood. Because the identification of specific

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Fig. 1. Binding of the PTP σ ectodomain to CSPG. (A) Domain structure of PTP σ and the CSPG neurocan. Δ Lys indicates the site in PTP σ where a cluster of lysines was mutated. (B to E) Purified recombinant PTP σ -Fc fusion protein was immobilized and treated with Ncn-AP fusion protein or AP tag control, followed by quantitation of bound AP activity. (B) Ncn-AP bound above control levels. (C) The PTP σ Δ Lys mutation reduced binding to background levels. Pretreatment of Ncn-AP with chondroitinase ABC (ChABC) reduced binding. (D) Binding between PTP σ -Fc and Ncn-AP was saturable. (E) Scatchard analysis produced a linear plot, indicating a single binding affinity with $K_D = 11$ nM. (F) PTP σ -AP bound to C8-D1A astrocyte cultures above AP control levels. Binding was reduced by antibody to CS (anti-CS), but not by an immunoglobulin M (IgM) control. (G) Chondroitinase ABC pretreatment of astrocyte cultures reduced PTP σ -AP binding. (H) PTP σ -Fc immunofluorescence (green) showed binding over cell surfaces (solid arrowhead) and extracellular matrix (open arrowhead); Fc control gave no visible fluorescence (not shown in the figure). Pre-incubation with anti-CS reduced binding. Blue is nuclear counterstain. (I) PTP σ -Fc, but not Fc control, coprecipitated neurocan from C8-D1A astrocyte lysates. Western blot showed bands with expected sizes for neurocan proteolytic fragments at approximately 150 and 100 kD (asterisks). *** $p < 0.001$, ** $p < 0.01$.

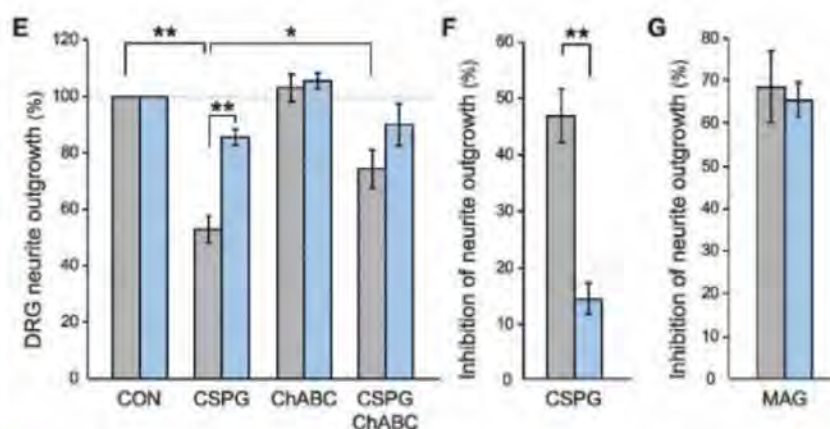
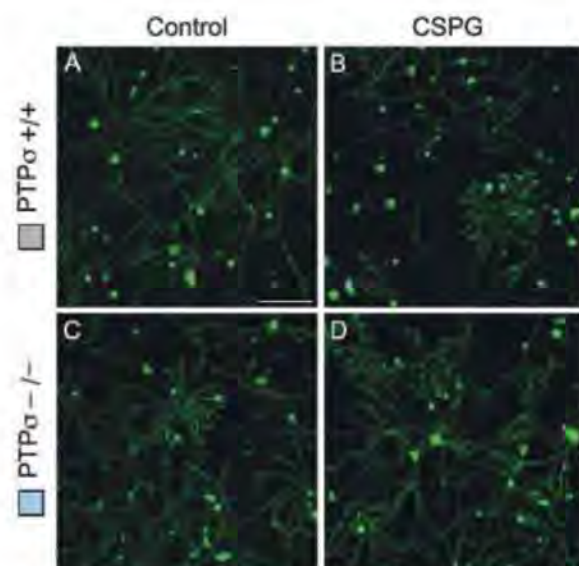
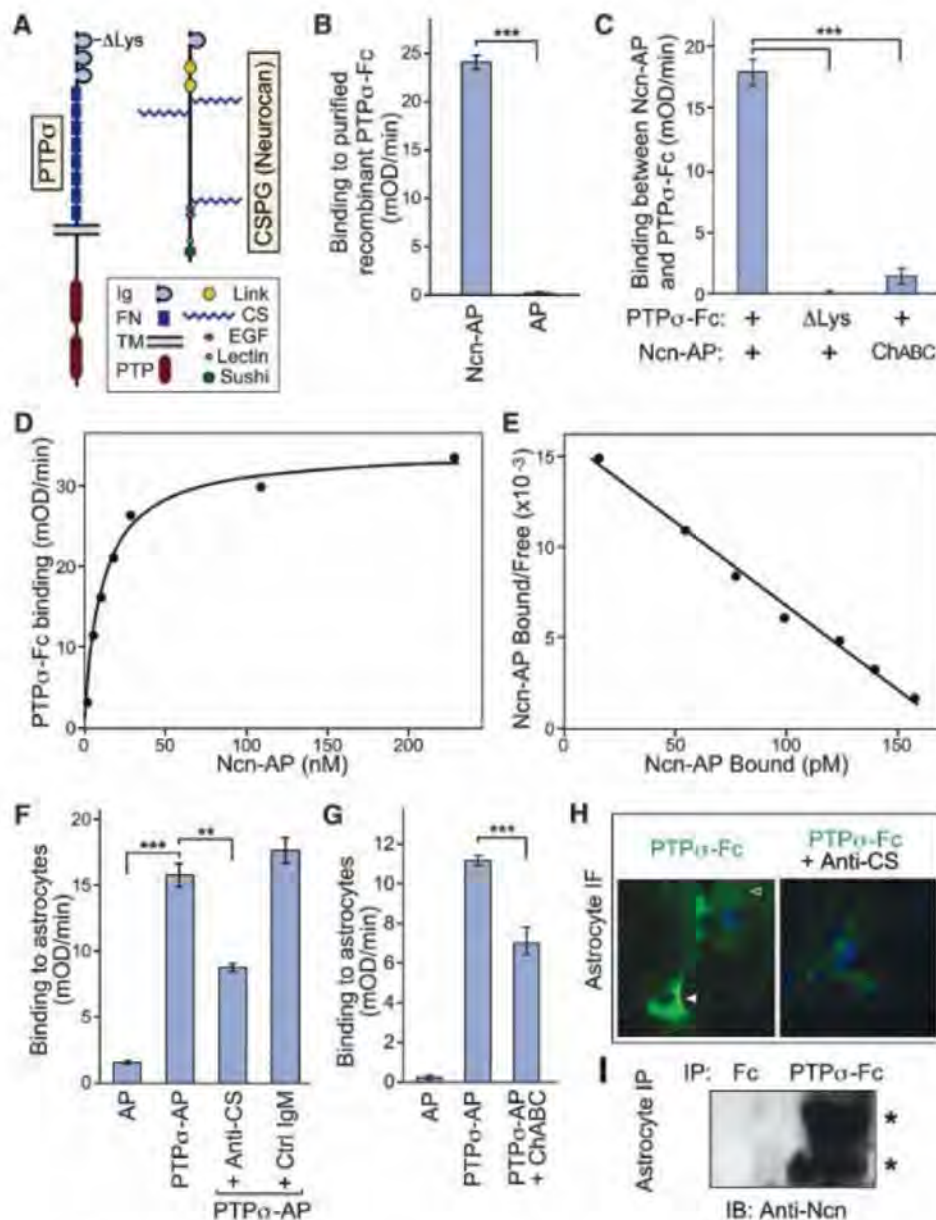


Fig. 2. Effect of PTP σ deficiency on the response of sensory neurons to CSPG. (A to D) DRG neurons from P8 mice were grown for 18 hours, then treated for 24 hours with or without CSPG and visualized by GAP-43 immunolabeling. (E) Quantitation of neurite outgrowth. PTP σ ^{-/-} neurons showed significantly less inhibition by CSPG than wild-type neurons did. A significant difference was no longer seen when chondroitinase ABC was added along with the CSPG. (F and G) Effect of CSPG or MAG, expressed as percentage of inhibition of outgrowth. PTP σ deficiency reduced the inhibitory action of CSPG but had no significant effect on the inhibitory action of MAG. $n = 5$ mice for each genotype. * $P < 0.05$, ** $P < 0.01$. Scale bars, 100 μ m.

neuronal receptors for CSPGs has been lacking, relatively nonspecific mechanisms brought about by arrays of negatively charged sulfate (16) or the occlusion of substrate adhesion molecules (17) have been suggested.

Transmembrane protein tyrosine phosphatases (PTPs) form a large and diverse molecular family and have a structure typical of transmembrane cell-surface receptors (18, 19). In previous work, we and others have found that PTP σ and other PTPs in the leukocyte antigen-related (LAR) subfamily can act as receptors for heparan sulfate proteoglycans (HSPGs) (20–22), and these PTPs are involved in axon guidance and synapse formation during development (18–22). In the adult, PTP σ gene disruption enhances regeneration in sciatic, facial, and optic nerves (23–25), although the mechanism for this enhancement has not been clear.

Finding that LAR subfamily PTPs are developmental receptors for HSPGs led us to investigate whether they might also be receptors for CSPGs. The CSPGs and HSPGs are analogous structurally, both consisting of a core protein bearing negatively charged sulfated carbohydrate side chains. To test for binding, we used PTP σ together with neurocan/CSPG3, a major CSPG that is produced by reactive astroglia after CNS injury

(26) (Fig. 1A). Using a cell-free system with recombinant fusion proteins of the PTP σ extracellular domain with an immunoglobulin Fc tag (PTP σ -Fc) and neurocan with an alkaline phosphatase tag (Ncn-AP), a binding interaction was indeed identified ($P < 0.001$) (Fig. 1B). Genuine biological ligand-receptor interactions are expected to show saturability and high affinity. Adding increasing amounts of Ncn-AP to PTP σ -Fc showed that the interaction is saturable (Fig. 1D), and Scatchard analysis produced a linear plot, indicating a single binding affinity with a dissociation constant (K_D) of approximately 11 nM (Fig. 1E). Binding was also demonstrated with aggrecan, another astroglial CSPG, producing a K_D of approximately 19 nM (fig. S1). These K_D s are similar to those we measured previously for the functional binding of *Drosophila* LAR to the HSPG ligands with which it interacts during development (22), and the values are within the typical nanomolar range for biologically significant ligand-receptor interactions.

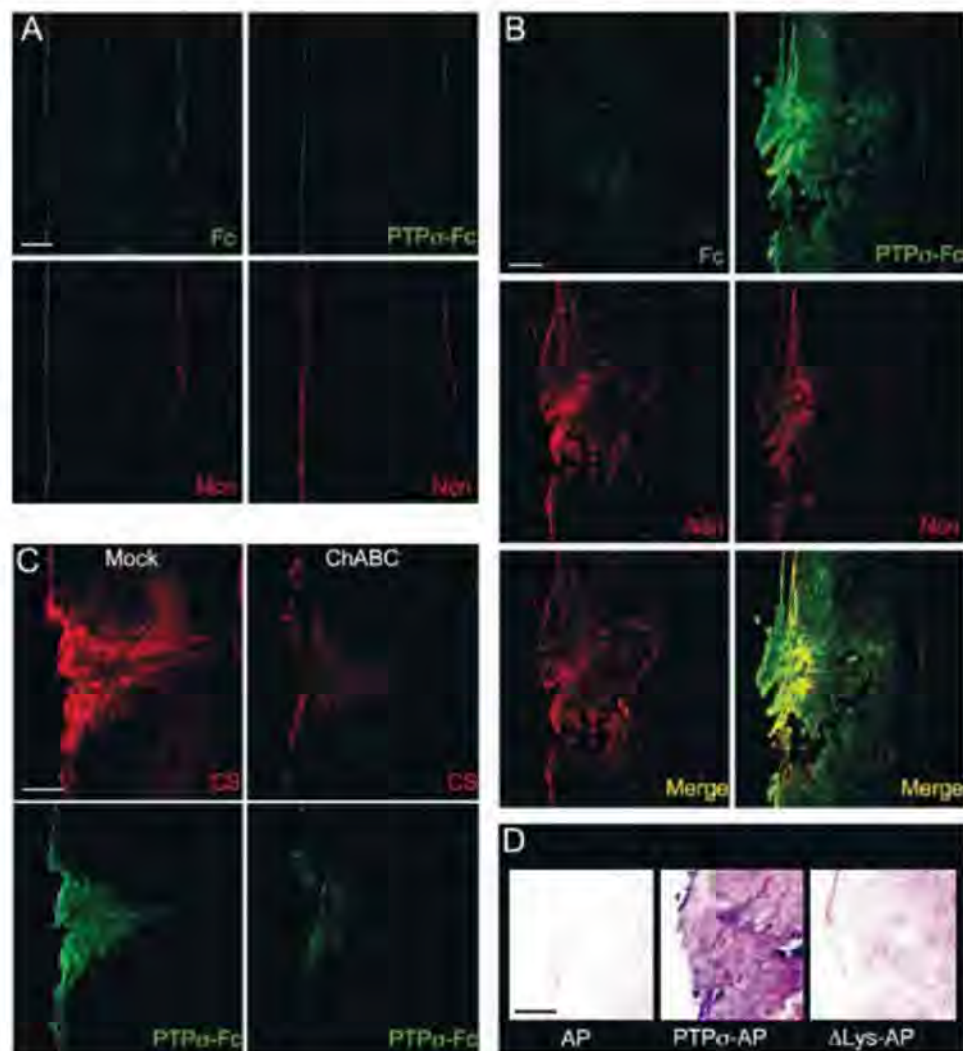
The CS moiety plays an important role in CSPG-mediated inhibition of neural regeneration (10, 12, 15, 16). We therefore tested whether the CS moiety of neurocan is involved in its interaction with PTP σ . Pretreatment of the Ncn-AP fusion protein with chondroitinase ABC abolished

most binding to PTP σ , confirming involvement of the CS chains ($P < 0.001$; Fig. 1C). Some binding remained, which might be due to incomplete digestion by chondroitinase ABC, which leaves a stub of CS. Other experiments showed that PTP σ binds to isolated CS chains (fig. S1). While it is possible that PTP σ might also interact with the core protein of CSPGs, these experiments indicate an involvement of the CS chains.

We also investigated the binding site on PTP σ . PTP σ has a conserved, positively charged region on the surface of the first immunoglobulin-like domain, and mutations of basic residues at this site impair binding of heparan sulfate (HS) (20). Because CS, like HS, is a negatively charged carbohydrate, it seemed plausible that this site might also bind CS. A cluster of four lysine residues in this domain, K67, K68, K70, and K71, were substituted with alanines (the Δ Lys mutant of PTP σ ; Fig. 1A). This substitution reduced binding to background levels ($P < 0.001$; Fig. 1C and fig. S1), identifying a CS interaction site on PTP σ .

To further address biological relevance, we examined whether PTP σ interacts with CSPG that is produced endogenously by astroglia, a cell type that produces inhibitory CSPGs at sites of neural injury. Because CS chains are added posttranslationally, using a relevant cell type could confirm

Fig. 3. PTP σ fusion protein detection of ligand distribution at a spinal cord lesion site. (A and B) Sections were double-fluorescence-labeled with antibody to neurocan (Ncn, red), together with either PTP σ -Fc probe (green) or Fc control (adjacent section, green). (A) Unlesioned spinal cord. Anti-neurocan labeled a thin line at the pia. PTP σ -Fc showed no labeling noticeably above Fc control levels. (B) Spinal cord 7 days after dorsal hemisection. The lesion site showed simultaneous elevation of neurocan immunolabeling and PTP σ -Fc binding. The distributions overlapped, with PTP σ -Fc labeling additional areas. (C) ChABC treatment reduced binding of PTP σ -Fc to the lesion site. Adjacent sections were preincubated with or without ChABC, then double-labeled with anti-CS (red) and PTP σ -Fc (green). (D) Introduction of Δ Lys mutation into a PTP σ -AP probe reduced lesion site binding (purple) close to AP tag control levels. Scale bars, 200 μ m.



binding with appropriately modified endogenous CSPGs. These experiments used mouse C8-DIA astrocytes, which express neurocan, display it on the cell surface, and deposit proteolytically processed neurocan fragments into the extracellular matrix (27). PTP σ fusion proteins were indeed found to bind astrocyte cultures, as shown by quantitative binding ($P < 0.001$; Fig. 1F) and immunofluorescence (Fig. 1H). Also, PTP σ -Fc coimmunoprecipitated neurocan fragments from astrocytes (Fig. 1I). The involvement of CS chains was confirmed by pretreatment of astrocytes with chondroitinase ABC or by pre-blocking with antibody to CS ($P < 0.01$; Fig. 1, F to H). These treatments did not eliminate all PTP σ binding, suggesting either that the treatments were only partially effective or that PTP σ may bind to molecular epitopes other than CS, such as keratan sulfate chains. In any case, the role of CS in this interaction makes it likely that PTP σ binds not only to neurocan and aggrecan but also to other CSPGs produced by astrocytes.

Having identified a binding interaction between PTP σ and CSPGs, we next tested whether PTP σ is functionally involved in the inhibitory effects of CSPG on neurons. Dorsal root ganglion (DRG) neurons express high levels of PTP σ throughout life (28). Postnatal day 8 (P8) DRG neurons from mice with a targeted gene disruption of PTP σ (29) or from wild-type controls were cultured in the presence of a neural CSPG mixture (Fig. 2) or purified neurocan (fig. S2). The CSPG mixture reduced control DRG neurite outgrowth by approximately 50% (Fig. 2, A, B, E, and F) but had far less effect on neurons from PTP σ ^{-/-} mice ($P < 0.01$; Fig. 2, C to F), showing a functional involvement of PTP σ in the response

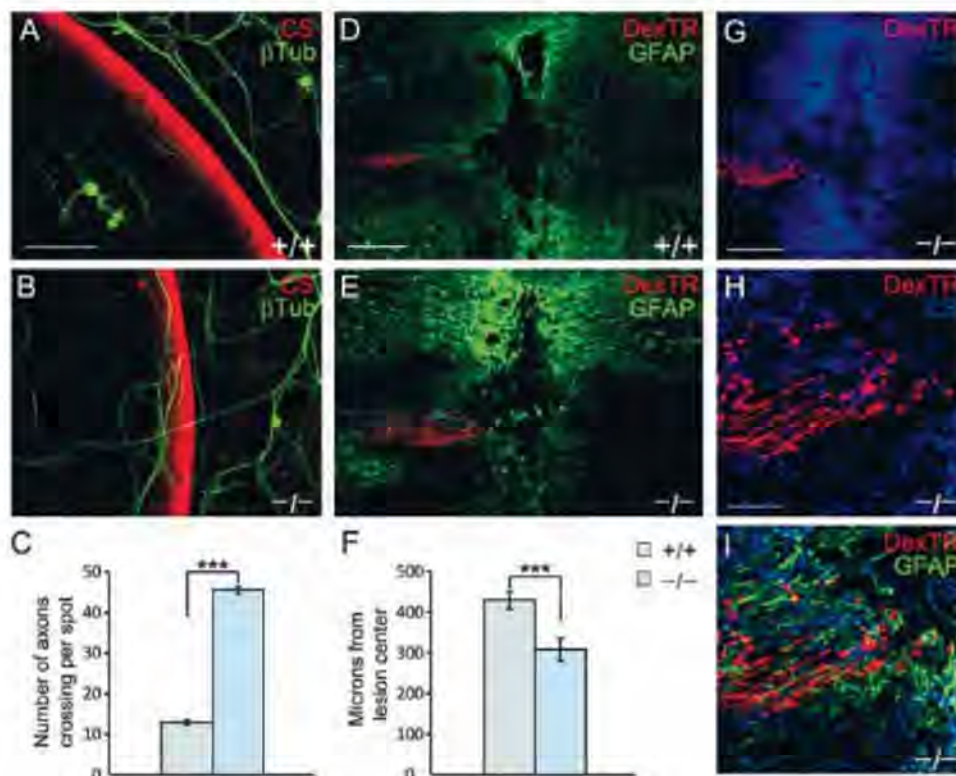
of young DRG neurons to inhibitory CSPGs. Comparable results were seen when neurons were challenged with purified neurocan ($P < 0.001$; fig. S2). The observation of some remaining inhibitory effect of CSPGs on PTP σ ^{-/-} neurons (Fig. 2F and fig. S2E) suggests the possible presence of additional receptors, which could be other PTPs in the LAR family or receptors in other families, or there could be additional receptor-independent mechanisms. To address the role of CS chains, the CSPG mixture was pretreated with chondroitinase ABC. This reduced its inhibitory effect on wild-type DRG neurons ($P < 0.05$) but did not cause a significant effect on PTP σ ^{-/-} neurons; thus, the outgrowth difference between wild-type and PTP σ ^{-/-} neurons was no longer statistically significant (Fig. 2E). This is consistent with our results showing that CS chains are involved in binding to PTP σ (Fig. 1 and fig. S1). Finally, we assessed whether the effect of PTP σ deficiency shows specificity for CSPG. We tested myelin-associated glycoprotein (MAG), a different inhibitory molecule (2), as well as nerve growth factor (NGF), which can oppose the effect of myelin-associated inhibitors (30). PTP σ deficiency did not affect neurite outgrowth in response to either MAG (Fig. 2G; $P = 0.75$) or NGF (fig. S3; $P = 0.67$ without NGF; $P = 0.99$ with NGF). Thus, PTP σ shows a specific functional role in the inhibitory response of DRG neurons to CSPG.

We next tested whether PTP σ has appropriate binding specificity to detect endogenous CSPG at sites of neural injury. In particular, we wanted to know whether PTP σ could preferentially recognize injured versus uninjured adult CNS tissue; this cannot be deduced simply from PTP σ 's ability to bind CSPGs (Fig. 1), because PTP σ also

binds HSPGs and, potentially, other ligands. Receptor ectodomain fusion proteins can be used to detect the distribution of their cognate ligands in tissues (31). PTP σ -Fc did not show obvious binding above background on uninjured spinal cord (Fig. 3A), whereas it showed strong binding around the lesion site 1 week after spinal cord injury (Fig. 3B). PTP σ -Fc labeling overlapped with neurocan immunolabeling, which was also induced by injury as expected (Fig. 3B), although the patterns were not identical. PTP σ -Fc appeared to label additional areas, which is consistent with binding to additional CSPGs. Binding was reduced by chondroitinase ABC treatment or by use of the PTP σ Δ Lys mutant (Fig. 3, C and D), once again demonstrating involvement of the CS moiety. These binding results on spinal cord provide further evidence for the role of PTP σ as a CSPG receptor and also show that it has appropriate binding specificity to serve biologically as a selective detector for injury sites in the adult CNS.

To test further for relevance to spinal cord injury, we used two functional models. After spinal cord injury, reactive glia produce an increasing gradient of CSPG beginning in the lesion penumbra and increasing toward the epicenter. In vivo, regenerating axons within this gradient stall and display dystrophic growth cone morphology (32). The dystrophic growth cone can be produced in vitro when neurons are exposed to a gradient of CSPG (8). We found a fourfold increase in PTP σ ^{-/-} neurites crossing the inhibitory outer rim of the gradient versus wild-type controls ($P < 0.001$; Fig. 4, A to C). We also used an in vivo spinal cord injury model, performing a dorsal column crush injury on adult mice and examining the position of labeled sensory axons in the

Fig. 4. Adult PTP σ ^{-/-} sensory neurons show reduced sensitivity to inhibition by a proteoglycan gradient and can extend further in a spinal cord lesion. (A and B) Adult wild-type DRG neurons visualized by antibody to β -tubulin (green) avoided the most inhibitory rim of the gradient visualized by anti-CS (red). PTP σ ^{-/-} neurons showed greater ability to cross the rim. (C) Quantitation of the average number of axons per spot growing up the gradient and crossing the outer rim. Wild-type, $n = 48$ spots; PTP σ ^{-/-}, $n = 40$ spots; *** $P < 0.001$. (D and E) Confocal images of longitudinal sections from adult mouse spinal cord 14 days after dorsal column crush, caudal to the left. Sensory axons are labeled with DexTR (red), and the lesion is delineated by glial fibrillary acidic protein⁺ (GFAP⁺) astrocytes (green). Wild-type axons are seen several hundred micrometers from the lesion center; PTP σ ^{-/-} axons about the edge of the lesion core. (F) Quantitation of distance from lesion center. Wild-type, $n = 35$ mice; PTP σ ^{-/-}, $n = 30$ mice. *** $P < 0.002$. (G to I) Confocal z-stack images of PTP σ ^{-/-} mouse dorsal column crush lesion, showing relationship between injured fibers (red), anti-CS labeling (blue), and in (I), reactive astrocytes (green). Scale bars in (A) and (B), 100 μ m; in (D) and (E), 200 μ m; in (G) to (I), 50 μ m.



fasciculus gracilis 14 days later. In the *PTPσ*^{-/-} mice, axon extension into the lesion penumbra was significantly improved ($P < 0.002$; Fig. 4, D to F, and fig. S4). *PTPσ*^{-/-} axons extended well into the region of inhibitory proteoglycan surrounding the lesion (Fig. 4, G to I). However, similar to the effect of chondroitinase (10), robust regeneration beyond the core of the lesion did not occur. This might, in principle, reflect partial redundancy with other PTPs in the LAR subfamily, and it would also be consistent with the known presence of other growth impediments such as the myelin inhibitors (1–5) and factors intrinsic to unconditioned neurons (33, 34). The results in this regeneration model system demonstrate a role for PTPσ in mediating the axonal response to the inhibitory CSPG-rich scar in a spinal cord lesion in vivo.

It has long been recognized that CSPG is one of the major inhibitors of neural regeneration; however, the mechanism has been poorly understood, and it has been unclear whether the mechanism even involves specific cellular receptors, limiting the options to tackle this important area by molecular approaches. Finding that PTPσ is a functional receptor that binds and mediates actions of CSPGs opens the door to new molecular approaches to understand CSPG action not only in regeneration, but also in development and plasticity. Our work on PTPσ also sheds new light on functions of the PTP family, and it will be interesting to know whether the other PTPs of the LAR subfamily may collaborate in nerve regeneration. The finding that a PTPσ fusion protein can detect lesion sites in the adult CNS not only sheds light on the biological role of PTPσ but also provides an injury biomarker, and thus a potential tool for research or diagnosis. Further-

more, the identification of a specific site on PTPσ that binds CSPG provides a lead for potential drug design to treat spinal cord injury. Alternative blocking approaches, such as soluble receptor ectodomains, could be used, and such approaches could potentially be combined with the blockade of other regeneration inhibitors. In addition to the possible treatment of spinal cord injury, the results here may be relevant to many other forms of neural injury as well as neurodegeneration that involves reactive astrogliosis. Identifying a functional receptor for a major class of regeneration inhibitors provides new pathways for research into mechanisms and therapeutic interventions to enhance regeneration or plasticity after nervous system injury.

Note added in proof: While this paper was in press, an additional characterization of the *PTPσ* gene knockout was published. Fry *et al.* (35) studied the corticospinal tract and reported regeneration after both surgical and contusive lesions, further contributing to the evidence in the present paper and previous studies that PTPσ acts in multiple areas of the nervous system and can play a key role in regeneration.

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Materials and Methods

Figs. S1 to S4

References

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Using Neural Measures of Economic Value to Solve the Public Goods Free-Rider Problem

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Every social group needs to decide when to provide public goods and how to allocate the costs among its members. Ideally, this decision would maximize the group's net benefits while also ensuring that every individual's benefit is greater than the cost he or she has to pay. Unfortunately, the economic theory of mechanism design has shown that this ideal solution is not feasible when the group leadership does not know the values of the individual group members for the public good. We show that this impossibility result can be overcome in laboratory settings by combining technologies for obtaining neural measures of value (functional magnetic resonance imaging-based pattern classification) with carefully designed institutions that allocate costs based on both reported and neurally measured values.

Public good allocation problems are pervasive in society. Examples in the government sector include the provision of national defense and environmental clean-ups. Examples in the private sector include hiring a

security guard or improving common areas in a condominium association. These examples highlight two key features of public goods. First, since their benefits are nonexcludable, they are enjoyed by all members of the group, even those

who do not help pay for them. Second, the optimal allocation of public goods depends on the group members' willingness to pay for them (1).

If the government (or group leadership) knew every individual's valuation for the good, the allocation problem would be straightforward: The government could compute the socially optimal level of the public good and then tax group members in proportion to the benefits that they receive in order to finance the cost of the good. In fact, in this case there are many possible fair rules for splitting the cost of the public good such that every individual's benefit from the public good is greater than his or her tax (2, 3). Unfortunately, individual valuations for public goods are not directly observable by the government, which makes the allocation problem challenging. In particular, self-interested individuals have an incentive to understate those values, if they are asked directly for their valuations and know that their share of the cost will increase with their reported values. This is known as the free-rider problem, and it makes it very difficult in practice to accurately determine which public goods should be provided and how the costs should be

shared. Countless experiments around the world have shown that the financial incentive to free-ride is pervasive and leads to allocations with a socially inefficient level of public good provision (4–6).

Social scientists have explored two different ways to limit the problems caused by free-riding. One approach investigates whether prosocial motives can be used to overcome the financial incentive to free-ride. For example, preplay communication and costly punishment of free-riders have been shown to ameliorate the problem in laboratory settings (7, 8). Although the full capabilities of these types of institutions are not yet known, the body of evidence (4, 5) suggests that prosocial motives are not always sufficient to eliminate free-riding behavior in all cultures (9). It is also unknown whether these motives are strong and pervasive enough to solve large-scale problems of practical interest.

The second approach has focused on designing institutions (known as “mechanisms”) that make it advantageous for self-interested individuals to reveal their true values. A mechanism is a set of rules specifying the information that is collected from the group members and how that information is used to decide how much of the public good to produce and how to split the costs. The number of potential mechanisms for public good problems is very large. Fortunately, the mechanism design problem is greatly simplified by a result, known as the revelation principle (10–12), which states that for every mechanism with a desirable set of properties, there is a related mechanism that achieves the same outcomes but in which individuals are simply asked to reveal their values. This result is useful and important because it limits the search space to direct revelation mechanisms: If a desirable solution does not exist within this class, then it does not exist at all.

A large body of work in economics has sought to design revelation mechanisms satisfying four desirable properties. The first is social efficiency (SE), which requires that the optimal amount of the public good always be produced, meaning that the net benefit to the group is maximized. The second property is dominant strategy incentive compatibility (DSIC), which requires that the wealth-maximizing strategy for each member of the group is to reveal his or her true value, regardless of others' values or behavior. This property is desirable because truthful reporting is essential for determining the socially efficient level of the public good, and DSIC ensures that every subject has a financial incentive to do so regardless of his or her beliefs about the other group members. The third property is balanced budget (BB), which requires that the cost of the

public good be completely covered by the members of the group. This property is desirable because it rules out the need for outside sources of funding. The fourth property is voluntary participation (VP), which requires that the expected value from participating in the mechanism be nonnegative for each individual, so that members do not have to be coerced into participating. A central result in economic theory is that there is no set of rules satisfying all four desired criteria (SE, DSIC, BB, and VP) simultaneously (13). In response to this fundamental impossibility result, theorists and experimenters have explored mechanisms that relax some of the criteria, but those mechanisms constitute a less than ideal solution to the problem (14, 15).

A key assumption behind the impossibility result is that the information used by the mechanisms is restricted to voluntarily reported values. However, a growing body of work in neuroscience has shown that it is possible to read subjective states with ranging degrees of accuracy (commonly 60 to 90%) using technology such as functional magnetic resonance imaging (fMRI) (16–23). This technology opens the door for a new class of mechanisms in which outcomes and payments depend both on individuals' reported values and on neural readings about their values. We refer to this new class of institutions as neurally informed mechanisms (NIMs).

To explore the technological feasibility of NIMs, we studied the public good allocation problem in a simple experimental setting. In each trial, subjects were randomly assigned to a group of size $N = 5, 10, 15, 20$, or 25 and were assigned either a low (\$0 to \$2) or high (\$8 to \$10) induced value for an abstract public good (24). The cost of this good was fixed at $\$5 \times N$. As is common in experimental economics, subjects were paid based on their payoffs in the experiment. Therefore, subjects were paid an amount equal to

their value for the public good if it was produced, and zero otherwise. Subjects made decisions in 50 different trials and were paid based on their average payoff from all trials. The overall payoff for each trial depended on the subject's value, the tax he or she had to pay (described below), and whether or not the public good was produced. Under the NIM, the public good was produced only when the sum of the reported values was greater than its cost. The true values were independently and identically drawn from a uniform distribution so that on average it was efficient to produce the public good in only half of the trials.

The experimental task procedure and rules of the NIM were as follows. First, subjects were shown the parameters of the decision problem in the sequential order depicted in Fig. 1A (24) while undergoing whole-brain fMRI. Their trial-specific value for the public good was shown in isolation during an initial screen, which allowed us to use a nonlinear support-vector-machine classifier (SVM) to predict subjects' values (high or low) based only on their pattern of neural responses to the value screen (24). After seeing the group size and the total cost of the public good, subjects chose whether to report their true value for the public good (high or low). If the public good was produced, the NIM then used both the classifier predictions and the reported values to determine the taxes paid by each individual, as depicted in Fig. 1B. Subjects are penalized with a higher tax when their reported value differs from the classifier's prediction. Furthermore, the higher the prediction accuracy, the more likely it is that a lie will be detected.

In the supporting online material (24), we show that the NIM satisfies SE, DSIC, BB, and VP. Because the public good is produced only when the reported values exceed the cost, SE requires that every individual reveal his or her true value. Subjects' incentives to reveal their true values depend

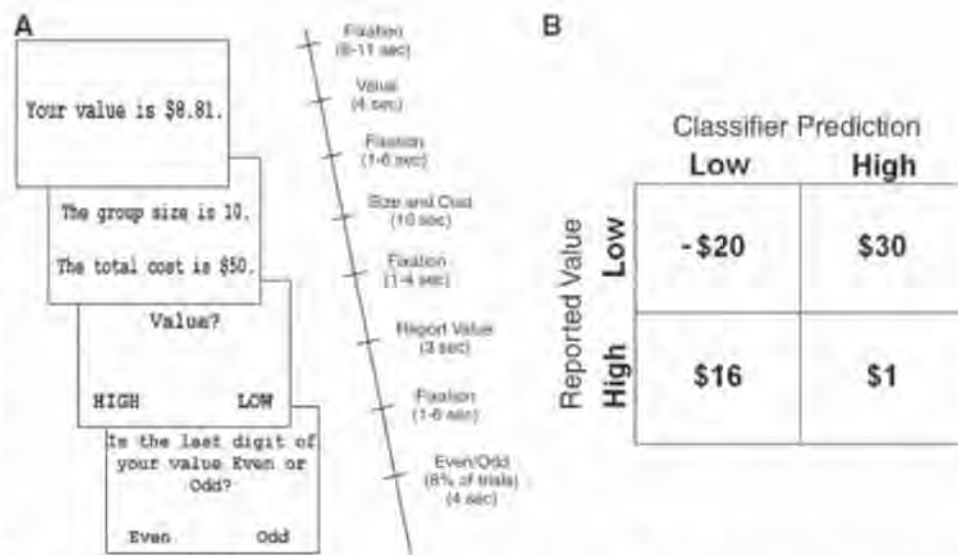


Fig. 1. (A) Timing of the experimental trials (top to bottom). (B) Tax paid by the subject in each trial as a function of the classifier's prediction and his or her reported type. Negative numbers denote transfers to the subjects.

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on what they believe the accuracy of the classifier to be. Figure 2A depicts the difference in expected payoff between truth-telling and lying as a function

of the classifier's accuracy. Low-value types are strictly better off revealing their true value for any classification rate between 50% (i.e., no decoding)

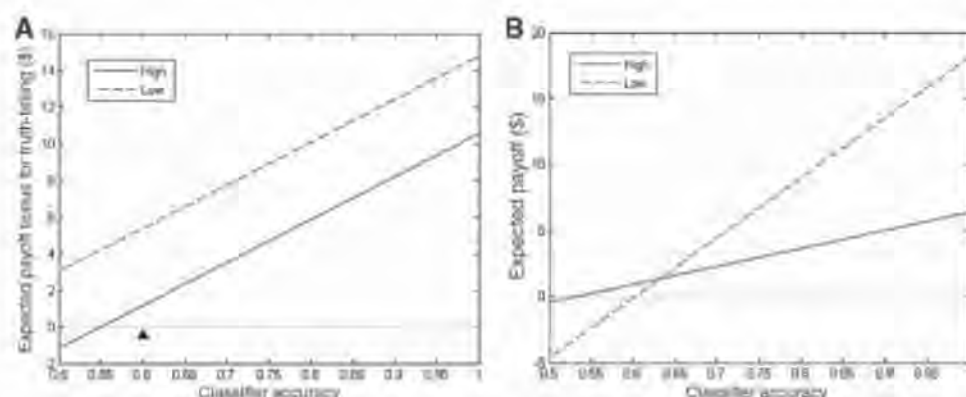


Fig. 2. (A) Expected benefit of truth-telling as a function of value type and classifier accuracy. For a particular classifier accuracy, the value of the curve indicates the difference in expected payoff between reporting truthfully and lying. Therefore, if the value is positive, then IC is satisfied and the subject should report his or her true value; if the value is negative, then IC is violated and the subject would earn more by misreporting his or her value. The arrow denotes the payoffs at the 60% accuracy rate used to describe the mechanism. A subject's decision is based on his or her beliefs about what the accuracy of the classifier will be and not on the realized accuracy after the experiment. (B) Total expected payoffs as a function of the actual classification accuracy of the mechanism for a subject who reveals his or her true type (24). For a particular classifier accuracy, the value of the curve indicates how much the subject can expect to earn, on average, if he or she reports his or her type truthfully. A positive value means that VP is satisfied; a negative value means VP is violated. Because the function is increasing with the accuracy rate, subjects have an incentive to cooperate with the experimenter to make the classifier as accurate as possible.

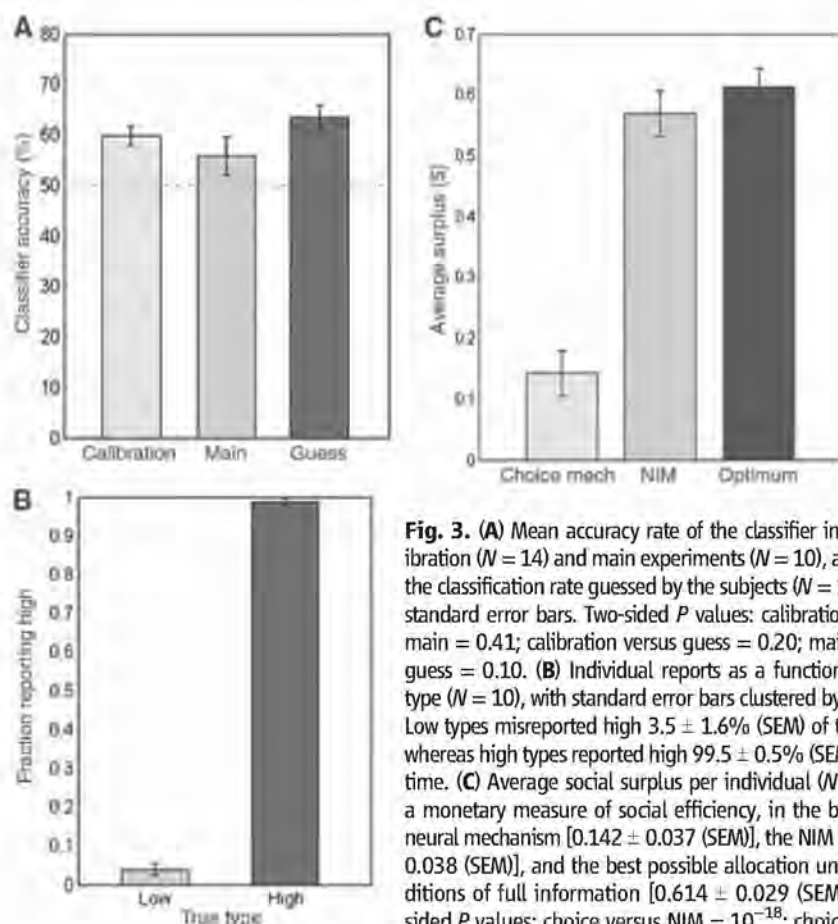


Fig. 3. (A) Mean accuracy rate of the classifier in the calibration ($N = 14$) and main experiments ($N = 10$), as well as the classification rate guessed by the subjects ($N = 10$), with standard error bars. Two-sided P values: calibration versus main = 0.41; calibration versus guess = 0.20; main versus guess = 0.10. (B) Individual reports as a function of true type ($N = 10$), with standard error bars clustered by subject. Low types misreported high $3.5 \pm 1.6\%$ (SEM) of the time, whereas high types reported high $99.5 \pm 0.5\%$ (SEM) of the time. (C) Average social surplus per individual ($N = 489$), a monetary measure of social efficiency, in the best non-neural mechanism [0.142 ± 0.037 (SEM)], the NIM [0.569 ± 0.038 (SEM)], and the best possible allocation under conditions of full information [0.614 ± 0.029 (SEM)]. One-sided P values: choice versus NIM = 10^{-18} ; choice versus optimum = 10^{-23} ; NIM versus optimum = 0.20.

and 100% (i.e., full decoding). In contrast, high-value types are strictly better off revealing their true value for any accuracy rate above 55% but have an incentive to lie for rates between 50% and 55%. This provides an intuition for why the mechanism satisfies DSIC, and thus SE, for classification rates above 55%. Figure 2B shows the total expected payoff from reporting truthfully in the NIM at different classifier accuracies, assuming that the other subjects are reporting truthfully and that they have high values 50% of the time. The expected payoff is positive for both value types at classification rates above 60%, which illustrates why VP is satisfied. Finally, BB is satisfied because by design the NIM distributes any financial surplus or deficit evenly between the players.

There was no feedback during the experiment, and subjects' values were classified afterward to determine their payments. Therefore, subjects made decisions based solely on their beliefs about the classifier's accuracy, which were assessed at the end of the experiment by debriefing. The rules of the NIM were explained to the subjects beforehand (24). In particular, they were told that in a previous experiment the same classification algorithm used here was able to predict values with an accuracy of 60%. Clear instructions about how the mechanism works are necessary to guard against comprehension mistakes, which would cloud interpretation of the results, and are considered a requirement by mechanism designers (25).

The $60 \pm 2\%$ (SEM) estimate for the classifier accuracy was based on an actual preliminary calibration experiment in which 14 subjects played a simple version of the NIM. In this experiment, the classified values played no role on outcomes and the subjects did not know that their values were being predicted (Fig. 3A) (24).

Figure 3 depicts the results of the experiment. The average classification accuracy was $56 \pm 4\%$ (SEM), insignificantly below the stated 60% rate (two-tailed $P = 0.33$) (Fig. 3A). We tested subjects' belief in the accuracy of the classifying technology by asking them to predict the classifier prediction rate for their own data and rewarding them based on the accuracy of their guess. During the debriefing period, subjects predicted a classification rate of $64 \pm 2\%$ (SEM), which is insignificantly different from the actual classification rate (two-tailed $P = 0.10$) (Fig. 3A). Most importantly, subjects revealed their true values nearly 100% of the time, consistent with the properties of the NIM at the subjects' predicted classification rates (Fig. 3B). Figure S15 shows that the frequency of truth-telling did not change during the experiment (24). Figure 3C compares the social surplus generated by the NIM, which is a measure of social efficiency, with two important benchmarks: (i) the social optimum that could be achieved if the government had full information and thus could always choose the socially efficient allocation and (ii) the theoretical average outcome generated by the best non-NIM mechanism satisfying BB, VP, and DSIC (24). The NIM generated 93% of the full-information so-

cial optimum, as compared with 23% for the best theoretical non-NIM mechanism.

This study establishes the viability of NIMs in a simple experimental setting with two types and with experimentally induced valuations. Because NIMs constitute a considerable departure from previous institutions used to solve the public goods allocation problem, it is worth highlighting several of their key properties.

First, NIMs advance the theory and practice of mechanism design by combining economic theory with neural measurement technology. In the past, economists have considered mechanisms that only use the reported values from each group member to determine whether the public good is produced and how the costs are shared. Here, we show that it is possible to do substantially better by also employing fMRI measures that are reliably correlated with value.

Furthermore, the use of NIMs is not limited to fMRI technology. As shown in detail in (24), all that is needed for the NIM to work is the existence of some signal of value that is known to be informative, whatever its source. Thus, simple physiological measures (e.g., pupil dilation or facial electromyography) might be feasible as well.

Another attractive property of NIMs is that they do not depend on beliefs about the types or behavior of the other group members. Truth-telling and voluntary participation are both dominant strategies with these mechanisms. The only requirement is that subjects believe that their values can be predicted with sufficient accuracy by the technology. Therefore, NIMs might not be viable if subjects could interfere with the technology. Fortunately, NIMs have a built-in incentive for subjects to make the classifier predictions as accurate as possible, because subjects' expected payoffs are increasing with the prediction accuracy (Fig. 2B).

Finally, VP is an attractive feature of the NIMs because it ensures that the public good makes every individual better off, so the entire group has an incentive to support the use of the NIM. Mechanisms are deliberately required to satisfy this VP property to bolster widespread acceptance. However, VP can be harder to satisfy when individuals have substantial amounts of risk or loss aversion (24), although the problem is substantially reduced as the accuracy of the neural measurements improves. Thus, future technological advances should alleviate this problem.

To summarize, the free-rider problem has been a challenge for economics, public policy, and political science since the work of Adam Smith (26). The field of mechanism design made substantial progress during the 20th century. Unfortunately, a major contribution of the theory was to show that an ideal solution is not possible when institutions rely only on revealed values. We have shown that this problem can be overcome in simple public good settings by using fMRI to obtain informative signals of individuals' values and using those signals to induce truthful reporting. Our results take the first step in combining physiological measurements with carefully designed mechanisms to create better institutions for collective decision-making. Future theory and experiments will be needed to take this technology to more practical applications.

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Materials and Methods

Figs. S1 to S15

Tables S1 to S3

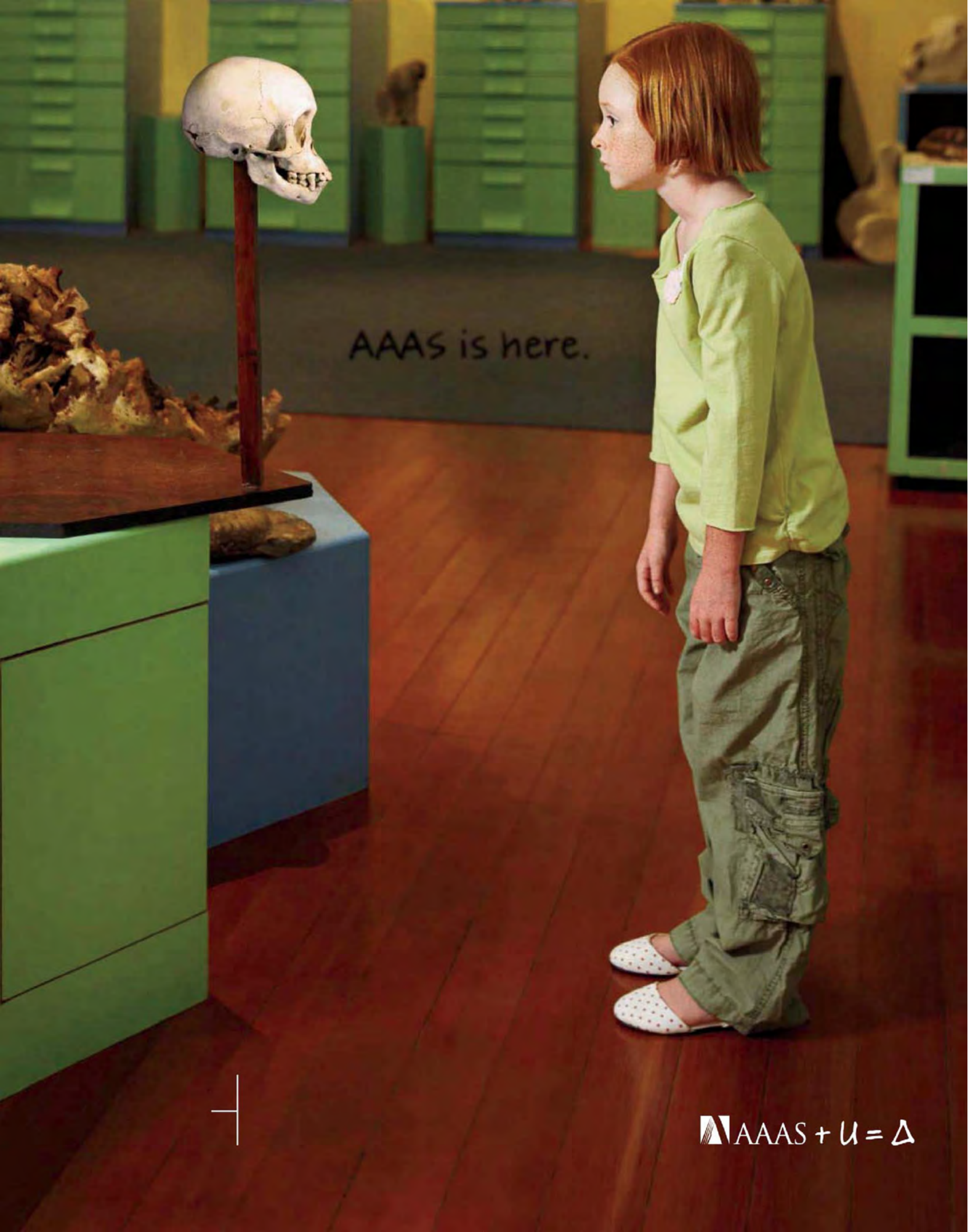
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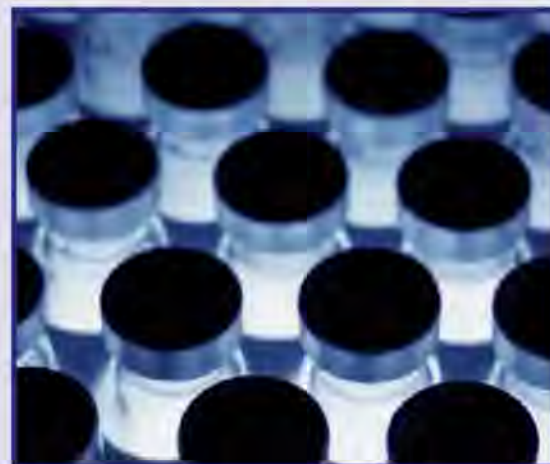
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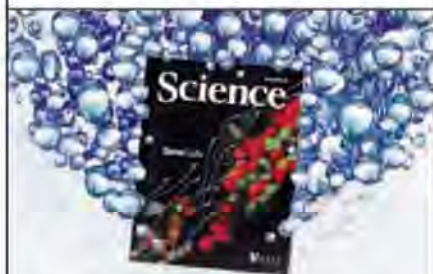
CircLigase II ssDNA Ligase efficiently catalyzes the circularization of single-stranded (ss) DNA greater than 15 bases without the need for oligonucleotide bridges. Virtually no linear or circular concatamers are produced by the enzyme. CircLigase II Ligase is an improved version of the original CircLigase enzyme, and is suitable for preparing circular templates from oligonucleotides or single-stranded complementary DNA for rolling-circle replication or rolling-circle transcription assays.

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Science Careers

From the journal Science



POSITIONS OPEN



FACULTY POSITIONS in Pharmaceutical and Biomedical Sciences College of Pharmacy University of Georgia

The Department of Pharmaceutical and Biomedical Sciences at the University of Georgia, Athens invites applications for two full-time, tenure-track faculty positions at the ASSISTANT or ASSOCIATE PROFESSOR levels in the areas of medicinal chemistry (apply for position #59842) and pharmacology/physiology (apply for position #59843). Qualified candidates should possess a Ph.D. or Pharm.D.-Ph.D. or equivalent degree with medicinal chemistry/bioactive natural products or pharmacology/physiology as the focus of their graduate education and research training. Excellent communication skills and the ability to teach at both the Pharm.D. and Ph.D. levels are required. Each successful applicant is expected to have or to develop a dynamic, extramurally funded research program in an area identified above. To be assured of full consideration, applications should be received by December 31, 2009. Interested qualified applicants should submit a letter of application, curriculum vitae, and a research plan and should have three confidential letters of recommendation sent to: Chair, Search Committee, Department of Pharmaceutical and Biomedical Sciences, R. C. Wilson Pharmacy Building, University of Georgia, Athens, GA 30602-2352. Applicants may also apply online to [e-mail: pbssearch@rx.uga.edu](mailto:pbssearch@rx.uga.edu). The University of Georgia is an Equal Employment Opportunity/Affirmative Action Employer. Applications from qualified women and minority candidates are encouraged.

ASSISTANT PROFESSOR, PHYSIOLOGIST Assistant Professor of Biology, Tenure Track Bridgewater State College Department of Biological Sciences

In search of a Physiologist who can support the biomedical concentration, is strongly committed to excellence in undergraduate teaching, in mentoring undergraduate research students and in advising, including the sharing of advising responsibilities for undergraduates interested in the biomedical and health professions. Teaching requirements will include human anatomy and physiology, introductory biology, and a first- or second-year seminar course. In addition, the applicant will teach an upper-level elective course, or courses, in the area of expertise that will complement and support the biomedical concentration. While the position is open to any field of biology, candidates with expertise in neurobiology or immunology are of special interest, but physiologists with expertise in other areas that complement our departmental offerings are also encouraged to apply. The successful candidate must have excellent communication skills and an earned Ph.D. by May 2010. Postdoctoral and teaching experience is preferred. Applicants should be strongly committed to excellence in teaching and advising, and to working in a multicultural environment that fosters diversity. They should also have an ability to use technology effectively in teaching and learning, the ability to work collaboratively, evidence of scholarly activity, and a commitment to public higher education. Salary commensurate with experience. Interested applicants should apply online at [website: http://jobs.bridgew.edu](http://jobs.bridgew.edu) and attach the following documents to their online application: a letter of interest, curriculum vitae, and statements of teaching and research. Review of applications will continue until the position is filled. Bridgewater State College is an Affirmative Action/Equal Opportunity Employer that actively seeks to increase the diversity of its work force.

POSITIONS OPEN



ASSISTANT PROFESSOR OF BIOCHEMISTRY. The Department of Chemistry and Biochemistry of the University of Arizona, in conjunction with UA's Department of Medicine and the Arizona Diabetes Program, seeks applications from Ph.D. scientists and/or M.D. clinicians for a tenure-track position in the general area of metabolic regulation. The newly merged Department of Chemistry and Biochemistry was awarded in excess of \$19 million in federal funding in 2008-2009, and it includes 45 plus faculty with broad research interests in the biological and chemical sciences. The laboratory space associated with the position is located in the new Medical Research Building that houses interdisciplinary basic biomedical research groups from several colleges, including the Arizona Diabetes Program. The successful candidate will be offered a competitive start-up package and will participate in teaching at the undergraduate, graduate, and/or medical student levels. To apply, please submit a letter of application, curriculum vitae, and statements of research and teaching interests online at [website: http://www.uacareertrack.com](http://www.uacareertrack.com) (position #43764). Three letters of recommendation should be sent to: Prof. Roger Miesfeld, Biochemistry Faculty Search, c/o Beth Vinson, Department of Chemistry and Biochemistry, P.O. Box 210041, Tucson, AZ 85721. Review of applications will begin November 6, 2009.

The University of Arizona is an Equal Employment Opportunity/Affirmative Action Employer. Minorities/Women/Persons with Disabilities/Veterans. Women and minorities are especially encouraged to apply. The Immigration Reform and Control Act requires you to have proof of authorization to work in the U.S.A.

ASSISTANT/ASSOCIATE PROFESSOR Environmental Studies

Goucher College invites applications for a new endowed chair in environmental studies. This is a tenure-track faculty position at the rank of Assistant or Associate Professor beginning August 2010.

We are seeking a natural scientist with strong interdisciplinary background in environmental science or environmental studies. Individuals with experience in global change research are preferred. Areas of expertise may include some combination of Earth science, geosciences, climate science, atmospheric science, or ecology. The individual will serve as Director of Environmental Studies, an interdisciplinary program spanning the social and natural sciences.

Teaching responsibilities include interdisciplinary core courses and upper-level courses in area of expertise. Competitive candidates should be able to establish a research program that includes undergraduate participation.

Ph.D. is required and postdoctoral experience is preferred.

Goucher College is a selective liberal arts college located in Towson, Maryland, 20 minutes north of Baltimore, Maryland. The college's strategic plan emphasizes environmental sustainability and international and intercultural experiences. There are opportunities for faculty to develop courses with an international focus.

Interested applicants must apply online at [website: http://goucher.interviewexchange.com](http://goucher.interviewexchange.com).

Please submit the following application materials online: (1) curriculum vitae; (2) letter of application; (3) statement of teaching and research interests.

Applicants should forward transcripts and three letters of recommendation separately to: Human Resources, Goucher College, 1021 Dulany Valley Road, Baltimore, MD 21204-2794. Review of completed applications will begin December 1, 2009.

Goucher College is an Equal Opportunity Employer and is committed to increasing the diversity of the campus community and encourages applicants that will fulfill that mission.

REAPING THE BENEFITS OF A GOVERNMENT JOB

Collectively, the US government is the largest single employer in America, filling approximately 1.6 million full-time, permanent positions; however, in reality, the US government consists of several hundred smaller employers, each of which has its own individual function and culture. As a result, the opportunities offered by government jobs are wide ranging, and the needs are diverse. **By Emma Hitt**

In times of economic hardship and downsizing, a science or engineering job with the US government is by comparison secure and accompanied by less pressure to obtain funding and grants than a job in academia. In addition, a lower risk of layoffs may exist there than in industry. Add to that the competitive benefits and salary, the learning opportunities, and the diverse working environments at various institutions, and a position with the US government can make an attractive proposition.

Christa Speekmann is an import/export specialist for farmed aquatic aquaculture at the US Department of Agriculture (USDA). Her job responsibilities include developing and implementing regulations for the importation of live animals into the United States. She also negotiates with foreign governments regarding health requirements for the export of live animals. "One benefit of working with USDA-APHIS [Animal and Plant Health Inspection Service] is using my science background to contribute to regulatory decisions, which are quickly implemented," she says. "Other advantages of working for the government include having job security and good retirement benefits," she says.

Peter Gehring, a physicist for the National Institute of Standards and Technology (NIST), researches "smart" materials such as lead-oxide ferroelectric relaxors and highly magnetostrictive compounds. "I feel very fortunate to be able to enjoy enormous freedom to pursue the research projects in which I am interested while working at a place that is scientifically vibrant and offers substantial job security comparable to that of academia," he says.

Barbara Thompson, an astrophysicist at the National Aeronautics and Space Administration's (NASA) Goddard Space Flight Center, works on space missions observing the Sun. "We have many dedicated scientists and engineers who form an extremely cohesive team," she says. "Most of us spend our entire careers here, so the commitment combined with the experience makes a very productive and exciting work environment," she adds.

Where and What Jobs

Any job of interest to a Ph.D. scientist is likely to be available in the US government. Positions exist in more than 2,000 separate job categories. The 1.6 million federal employees are distributed among jobs in 15 cabinet-level agencies; 20 large, independent agencies (>1,000 employees); and 80 small agencies (<1,000 employees), according to a 2007 report compiled by the Partnership for Public Service. Contrary to popular belief, over 80 percent of government jobs are located outside of the Washington, D.C., metropolitan area, in locations across the United States (largest concentrations include Baltimore, Philadelphia, Atlanta, San Diego, and New York City) and worldwide. The health care occupations and sciences are projected to make up some of the fastest growing areas. *continued »*



Denise Koo



Paul Sandifer

“Other advantages of working for the government include having job security and good retirement benefits.”

UPCOMING FEATURES

Reentering the Work Force (Online only) — November 27

Focus on Denmark — January 15

Diversity 1: Women in Science — February 12

JOBS IN GOVERNMENT

“It’s amazing the things that we have done that have an impact on people’s everyday lives.”

—Sandy Miller Hays



Information Resources

The first step toward seeking a job with the US government is to search the federal jobs website www.USAJobs.gov. This website includes a database of nearly all federal jobs, searchable by keyword, location, income level, and other parameters. At the time of this writing, the database included about 34,000 opportunities worldwide. A search with the word “scientist” pulls up over 700 jobs, and a search with the word “biology” pulls up over 100 listings (42 that pay over \$100,000). In addition to the actual listings, the USAJobs website has a wealth of information about US government jobs, including a listing of the locations that are hiring the most people, the occupations most in demand, and the agencies with the most openings. Links to the webpages of the human resources departments for each of the individual agencies are also available.

Another website, www.bestplacestowork.org, describes the most highly rated places to work within the federal government, and represents a compilation of surveys from more than 200,000 federal employees. Currently, the Nuclear Regulatory Commission takes the top position based on several factors, including employee skills-mission match, effective leadership, training and development, family-friendly culture, pay and benefits, and work/life balance. The National Science Foundation is No. 5 out of 32 smaller agencies, and NASA is No. 3 and the Environmental Protection Agency is No. 6 out of 30 large agencies. The Department of Health and Human Services, which encompasses the National Institutes of Health (NIH), ranks 21st out of 30. The data available on this website can also be configured to help job seekers find the most appropriate agency based on select criteria.

The Office of Personnel Management (OPM) website (www.opm.gov) includes information on federal benefits and salaries as well as the demographics of the federal work force. A subdomain of that website, FedScope (www.fedscope.opm.gov), also provides statistics such as demographic breakdowns by state, department, and/or type of position. Given that so much information is available, it is possible to thoroughly assess a position or institution before applying for a federal job.

Getting Specific

In addition to the USAJobs website, each of the individual agencies has specific websites and ways to navigate opportunities within an institution. “Right now we have about 110 postdoctoral fellowships being advertised at our training website (www.training.nih.gov),” says **Roland Owens**, chief recruiter for the NIH intramural research program. “We usually also encourage people to make direct contact with a principal investigator if they are looking for a postdoctoral position,” he adds. Owens recommends that contact with a prospective principal investigator about a postdoctoral position should be made at least six months before the desired start date.

An effective way to find out information on postdoctoral positions at the NIH, Owens says, is to go to the annual report listings at intramural.nih.gov/search/index.html. At that webpage, the annual reports can be searched by keyword. For example, typing in the key word “sarcoma” will pull up a listing of principal investigators at the NIH studying sarcoma—with a brief description of their research—who may have available appropriate postdoctoral opportunities.

Within the National Oceanic and Atmospheric Administration (NOAA), again, all the jobs can be found posted at the website USAJobs.gov, but **Paul Sandifer**, senior science adviser to the NOAA administrator, suggests that job seekers should contact the human resource offices at NOAA facilities in the area and talk to current employees, “particularly those working in doctoral positions in the agency. They should also consult academic mentors who are knowledgeable about the NOAA work force and areas for which we are seeking employees.”

“For people without much experience, taking on a fellowship is one of the best ways to get a foot in the door,” says **Denise Koo**, acting director of the Centers for Disease Control and Prevention (CDC) Office of Workforce and Career Development. “There’s an emerging infectious disease laboratory fellowship and an American Society for Microbiology laboratory fellowship, which generally last one to two years,” she adds. “A fellowship gives you the opportunity to get to know what the jobs are, and also obtain the public health experience that will help during the application process.”

Exacting Standards

Regardless of the implications of that old quip “close enough for government work,” the standards of science and work ethic compare well with those of academia and industry. The CDC’s Koo says that “in some ways, science at the CDC is perhaps more rigorous because it gets examined so much,” she says. “We’re government, and so we have a responsibility to be putting out recommendations and scientific data that are reliable—we have a whole peer-review process, and you’re not even allowed to publish anything until it goes through that process at the agency,” she says. **continued »**

Featured Participants

Centers for Disease Control and Prevention
www.cdc.gov

Department of Agriculture
www.usda.gov

Department of Health and Human Services
www.hhs.gov

Environmental Protection Agency
www.epa.gov

National Aeronautics and Space Administration
www.nasa.gov

National Institute of Standards and Technology
www.nist.gov

National Institutes of Health
www.nih.gov

National Oceanic and Atmospheric Administration
www.noaa.gov

National Science Foundation
www.nsf.gov

Nuclear Regulatory Commission
www.nrc.gov

Office of Personnel Management
www.opms.gov



NIH Intramural Research Program is Recruiting “Earl Stadtman Investigators”



The National Institutes of Health, the nation's premier agency for biomedical and behavioral research, is pleased to announce the launch of its search for top-tier tenure-track candidates to become “NIH Earl Stadtman Investigators.”

Earl Stadtman was an outstanding NIH scientist who mentored many current leaders in the biomedical community. In his honor, the NIH is recruiting basic, clinical and population-based investigators who seek the flexibility of scientific exploration in an intellectual and supportive environment. NIH investigators have the ability to focus on research that is high risk; they can quickly redeploy resources to explore new directions, collaborate freely on multidisciplinary teams, and contribute to the NIH mission of improving human health. The receipt of prestigious awards and honors—Nobel prizes, Lasker awards, elections to the NAS and IOM, among others—demonstrates the scientific rigor of our NIH intramural investigators.

We offer competitive startup packages and a collaborative, academic environment with more than 1,100 principal investigators engaged in cutting-edge basic, translational, clinical and population-based research. Our scientists focus entirely on their research with ample opportunities to mentor and train outstanding fellows at all levels. One special feature of our research program is the NIH Clinical Center, the world's largest hospital entirely devoted to biomedical research.

Qualifications/Eligibility: Candidates must have an M.D., Ph.D., D.D.S./D.M.D., D.V.M, D.O. or equivalent doctoral degree and have an outstanding record of research accomplishments as evidenced by publications in major peer-reviewed journals. Preference will be given to applicants who are in the early stages of their research careers. Candidates in any area of biomedical, clinical and behavioral research are invited to apply. Appointees may be U.S. citizens, resident aliens or non-resident aliens with, or eligible to obtain, a valid employment-authorized visa.

Salary: Successful candidates are offered competitive salaries commensurate with experience and qualifications, and they are assigned ample research space, supported positions and an operating budget.

To Apply: Complete applications should be received by November 1, 2009; however, applications will be accepted until available positions are filled. Interested applicants should submit a curriculum vitae, a three-page description of proposed research, and three letters of recommendation through our online application system, at <http://tenuretrack.nih.gov>. No paper applications will be accepted.

For information on the NIH Intramural Research Program, refer to <http://intramural.nih.gov/search> and <http://www1.od.nih.gov/oir/sourcebook/sci-prgms/sci-prgms-toc.htm>. Specific questions regarding this recruitment effort may be directed to Dr. Roland Owens, Assistant Director, NIH Office of Intramural Research at owensrol@mail.nih.gov.

We seek original and interactive thinkers to be part of the next generation of research leaders. Become part of a team that continues to make history.

DHHS and NIH are Equal Opportunity Employers.



**Department of Health and Human Services
National Institutes of Health
Intramural Research Programs
NCI, NEI, NHLBI, NIAID, NIDDK, NIMH, NINDS and CIT
Bethesda, MD**



**Tenure Track/Tenured Positions
In Systems Biology**

The NIH Intramural Research Program (IRP) is recruiting outstanding systems biologists at the tenure-track or tenured levels. These individuals will direct independent research programs on the NIH campus in Bethesda, MD and participate in a Trans-NIH Initiative in Systems Biology promoting interaction between experimentalists, theoreticians and computational investigators. Candidates will have demonstrated an ability to conduct outstanding independent biomedical research on key topics in systems biology such as computational modeling of biological processes at various scales, analysis of global datasets, construction and analysis of biological networks, and 'omic' scale interrogation of biological systems. The internationally recognized NIH faculty covers a wide range of basic and clinical research topics with a growing strength, support and emphasis on systems biology and informatic approaches to biomedicine.

The NIH IRP promotes creative and innovative science unconstrained by the conventional support mechanisms demanded at academic or private research institutes. Investigators have ready access to and support from state-of-the art experimental and computational cores and facilities, and a variety of programs to recruit graduate students and post-doctoral fellows.

Candidates must have an M.D. and/or Ph.D., or equivalent doctoral degree, and an outstanding record of research accomplishment and peer-reviewed publications. Recruits will be provided a competitive salary commensurate with experience and qualifications, and will be assigned ample research space, supported positions, operating budget, and start-up funds. Appointees may be US citizens, resident aliens, or eligible foreign nationals. Review of applications will commence on Nov. 1, 2009 and continue until the positions are filled. Please submit a curriculum vitae, brief (not to exceed 3 pages) statement of research interests that includes how you see your research group helping to create a world-class, integrated systems biology effort at NIH, and three letters of reference in .pdf or MS word format only (no paper applications will be accepted) to: <http://tenuretrack.nih.gov/apply/>



Physician-Scientist Investigator Recruitment National Human Genome Research Institute

The Genetic Disease Research Branch (GDRB) of the National Human Genome Research Institute (NHGRI) provides unparalleled opportunities for investigators to develop world-class research programs in genetics and genomics. The Branch is pleased to announce that it is seeking to recruit a new tenure-track investigator to pursue innovative, independent research as part of this group of highly interactive and supportive investigators.

The successful candidate should have an interest in developing a research program that integrates clinical research with genetic or genomic approaches to understand the mechanisms of developmental processes or human disease. We welcome applications from clinicians with a wide range of backgrounds and disciplines.

Current GDRB faculty members use a variety of approaches to study the regulation and function of genes involved in development and homeostasis in humans and model organisms, with the goal of providing insight into human diseases. We welcome applicants with human disease-oriented research interests and approaches that complement those of our current Branch faculty.

The successful candidate will take advantage of interactions with a highly collegial group of scientists within NHGRI and on the NIH campus as a whole. In addition, they will have access to NHGRI's outstanding core laboratories and the unparalleled resources of the NIH Clinical Center. This position includes a generous start-up allowance, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees.

Candidates should have an M.D., an M.D.-Ph.D., or equivalent degree, as well as advanced training and a record of accomplishments. Candidates with Ph.D.s who have experience organizing or supervising integrated basic and clinical research projects are also eligible. Such candidates should contact the Search Committee Chair or Branch Chief prior to submitting a formal application. Interested applicants should submit their *curriculum vitae*, a three-page description of proposed research, and three letters of recommendation through our online application system, at <http://research.nhgri.nih.gov/apply>.

Applications will be reviewed starting Monday, November 23, 2009 and will be accepted until the position is filled.

For more information on GDRB and NHGRI's Intramural Program, please see <http://genome.gov/DIR>. Specific questions regarding the recruitment may be directed to Dr. William Pavan, the Search Chair, at bpavan@nhgri.nih.gov. Questions may also be directed to Dr. Leslie Biesecker, the GDRB Branch Chief, at leslieb@helix.nih.gov.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR





WWW.NIH.GOV

Tenure-Track/Tenured Investigator Laboratory of Immunology

The Laboratory of Immunology (LI), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) invites applications for a tenure-track/tenured investigator position in immunology. Applicants should have a Ph.D., M.D., or equivalent degree; an outstanding record of postdoctoral accomplishment; and an interest in any area of biomedical research related to immunology.

Specifically, we seek a highly creative individual who will establish an independent, world-class research program that takes full advantage of the special opportunities afforded by the stable, long-term funding of the intramural research program at NIH. She or he should be interested in developing and applying novel approaches to the study of problems of major biological and/or medical importance, which could include a significant clinical or translational effort in addition to bench research. In the former case, the successful candidate would have access to the NIH Clinical Center, a state-of-the-art research hospital on the NIH campus in Bethesda, MD, and ample opportunity to participate in the activities of the Trans-NIH Center for Human Immunology.

Generous ongoing support for salary, technical personnel, postdoctoral fellows, equipment, and research supplies will be provided. Available core or collaborative facilities include flow cytometry, advanced optical imaging, microarray generation and analysis, high throughput sequencing, computational biology, production of transgenic and gene-manipulated mice, biosafety level (BSL)-3 facilities, chemical genomics, and support for projects involving RNAi screening. The successful applicant will also have access to Trans-NIH initiatives involving technology development, translational investigation, and multidisciplinary science. In addition to an outstanding international postdoctoral community, a superior pool of graduate and undergraduate students is available to the successful applicant.

LI has a distinguished history of accomplishment in immunology. We strongly encourage application by outstanding investigators who can continue and enhance this record of achievement. Current LI investigators are Ronald Germain, Michael Lenardo, David Margulies, Stefan Muljo, William Paul, Ethan Shevach, and Tsan Xiao.

To apply, e-mail curriculum vitae, bibliography, and outline of a proposed research program (no more than two pages) to Ms. Yushekia Hill at NIAID.DIR.Search@niaid.nih.gov. In addition, three letters of reference must be sent directly from the referee to Drs. Giorgio Trinchieri and Dan Kastner, Co-Chairs, NIAID Search Committee, c/o Ms. Yushekia Hill, at NIAID.DIR.Search@niaid.nih.gov or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, MD 20892-1356. E-mail is preferred. Applications will be reviewed starting **11/16/09** and will be accepted until the position is filled. Please refer to ad #028 on all communications. For further information about this position, contact Dr. William Paul at 301-496-5046 or wpaul@niaid.nih.gov.

A full package of benefits (including retirement and health, life, and long-term care insurance) is available. Women and minorities are especially encouraged to apply. U.S. citizenship is not required.

National Institute of Allergy and Infectious Diseases

To learn more about NIAID and how you can work in this exciting research organization, please visit us on the web at www.niaid.nih.gov/careers/sti.



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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National Institute of Allergy and Infectious Diseases

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NIAID

National Institute of Neurological Disorders and Stroke



Tenure-Track Investigator Basic Research in Neuroscience Division of Intramural Research

The Division of Intramural Research of the National Institute of Neurological Disorders and Stroke, NIH is searching for outstanding individuals for tenure-track positions in areas of basic research of neuroscience. The Division of Intramural Research of NINDS has basic research programs to support the new applicant in neurodevelopment, neural circuits, synaptic function, membrane protein biophysics, biological basis of neurodegenerative diseases, and neuroimaging. These programs operate in one of the largest and most active neuroscience research communities in the world, which has been integrated in the Porter Neuroscience Research Center. In addition there are active clinical programs in neurological disorders and stroke. The successful individual will be expected to develop and direct an independent research program focused on relevant problems in the neurosciences. Outstanding individuals interested in neurodevelopment, neural circuit function, and mechanisms of neurodegeneration are particularly encouraged to apply. Candidates that make use of state-of-the-art techniques in stem cell biology or genomic/systems biology to study problems in the neurosciences are also encouraged to apply. The candidate will have earned a Ph.D. degree or MD or equivalent and will have demonstrated the potential to become an outstanding independent investigator. In rare cases outstanding senior candidates will be considered for a tenured position if there is a demonstrated international reputation and well-documented evidence of ongoing independent accomplishments. An individual selected for a tenure-track position is expected to build a dynamic and productive research group. Laboratory space and start-up funds, access to shared research facilities, on-going research support and salary are competitive with premier academic institutions. Applicants should send curriculum vitae, bibliography, statement of research interests, and have three letters of reference sent to: **Alan Koretsky, National Institute of Neurological Disorders and Stroke, c/o Nhuyen Quach, Office of the Scientific Director, Division of Intramural Research, Building 35 Room GA908, NIH, Bethesda, MD 20892 or to nindsbasicsearch@ninds.nih.gov**. Applications will be reviewed beginning **December 1, 2009** and proceed until successful candidates are recruited.

National Institute of Neurological Disorders and Stroke



Tenure-Track Investigator Clinical/Translational Research Division of Intramural Research

The Division of Intramural Research of the National Institute of Neurological Disorders and Stroke, NIH is searching for outstanding clinician-investigators for tenure-track positions in the area of clinical/translational studies of neurological disorders or stroke. The Division of Intramural Research boasts active clinical research programs in Stroke/Traumatic Brain Injury, Movement Disorders, Neuroimmunology, Neurogenetics, Cortical Plasticity relevant to neurological disorders and stroke, Surgical Neurology and Neuroimaging. These programs operate in one of the largest and most active clinical research environments in the world, making use of the Clinical Research Center at NIH. They also interact with a large and active basic neuroscience community which has outstanding programs in ion channel biophysics, synaptic physiology, neural circuit function, cell biology, and developmental biology. The successful individual will be expected to develop and direct an independent research program focused on clinical or translational problems that relate to the major areas of interest in the intramural program. The successful candidate will also be expected to develop strong interactions with one of the active clinical/translational programs. The individual should have a demonstrated background and knowledge in research focused on diseases of the nervous system. Experience in application of clinical trial methodology to the study of disease mechanisms and testing new therapies is highly desirable. The candidate will have earned a M.D. or M.D./Ph.D. degree and will have excellent scientific skills in structuring an original and productive research program using outstanding communication and collaborative abilities. Preference will be given to individuals who have a medical license in the United States, who have completed training in an accredited training program in neurology, and are either board eligible or board certified. Outstanding candidates may also be considered for a tenured position if there is a demonstrated international reputation and well-documented evidence of ongoing independent accomplishments. An individual selected for a tenure-track position is expected to build a dynamic and productive research group. Laboratory/clinical facilities, shared research facilities, research funds and salary are competitive with premier academic institutions. Applicants should send curriculum vitae, bibliography, statement of research interests, and have three letters of reference sent to: **Kenneth Fischbeck, National Institute of Neurological Disorders and Stroke, c/o Nhuyen Quach, Office of the Scientific Director, Division of Intramural Research, Building 35 Room GA908, NIH, Bethesda, MD 20892 or nindsclinicalsearch@ninds.nih.gov**. Review of applications is expected to begin on November 30, 2009, but applications will be accepted until the position is filled.



WWW.NIH.GOV



The Cell and Cancer Biology Branch (CCBB), CCR, National Cancer Institute has proven strength in molecular biology of carcinogenesis, metastasis, cell signaling and apoptosis. Current Principal Investigators can be found at the following website: <http://ccr.cancer.gov/labs/lab.asp?labid=80>. The CCBB now invites applications for a tenure track investigator to establish an outstanding independent basic and/or translational research program in a critical area of cancer biology, including inflammation and the cancer microenvironment, cancer stem cells, or metastasis. An adjunct clinical appointment might be possible for individuals with appropriate clinical training. Candidates must have a Ph.D. and /or M.D. and a proven record of innovative research and productivity in cancer related biological processes. Applicant will be provided sufficient support for personnel, space, equipment and supply budget. Salary is competitive and commensurate with research experience and accomplishments and a full civil service package of benefits.

Review of applications will begin on November 15, 2009 and the search will be closed on January 15, 2010. Interested individuals should send a cover letter, curriculum vitae, a brief summary of research experience, accomplishments and future plans, copies of five publications, and three letters of recommendation to: Mrs. Laverne McLean, Department of Health and Human Services, National Cancer Institutes of Health, 37 Convent Drive, Room 1068, Bethesda, Maryland 20892, Tel: 301-435-4652; Fax: 301-435-4655 E-mail: mcleanl@mail.nih.gov. DHHS and NIH are Equal Opportunity Employers.



Postdoctoral Fellows: Protein Expression and X-ray Crystallography

The Macromolecular Crystallography Laboratory (MCL), Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH) is seeking applications for postdoctoral fellowships from exceptional candidates for 2 of their Sections of the MCL. Dr. Jacek Lubkowski is seeking a candidate with a recent Ph.D. degree and a strong publication record in the areas of gene cloning, protein expression in eukaryotic systems, and protein purification. Dr. Lubkowski's research focuses on the structural properties of Janus kinases, members of the TREM family of receptors, and defensins; and correlating these properties with biological functions. Dr. David S. Waugh is seeking either a candidate with similar qualifications who is interested in leading an effort to develop novel technology for the production of recombinant proteins in eukaryotic organisms or a candidate who is already an accomplished protein crystallographer and who wishes to continue working in this field while also learning cutting-edge techniques for the production of recombinant proteins and protein engineering. Dr. Waugh's research interests revolve around methods development for the production of recombinant proteins, X-ray crystallographic studies of virulence factors from potential agents of bioterrorism, and structure-assisted development of therapeutic agents for the treatment of cancer. MCL is equipped with state-of-the-art X-ray equipment and complete infrastructure for the preparation and characterization of functional recombinant proteins. Additional support is available from a broad range of core services. US citizenship is not required but proficiency in English is essential. Please submit curriculum vitae including contact information for three references via e-mail to Dr. Lubkowski (lubkowsj@mail.nih.gov) or Dr. Waugh (waughd@mail.nih.gov).

Salary is commensurate with research experience and accomplishments. With nationwide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health.

The NIH Director's Wednesday Afternoon Lecture Series

Biomedical scientists around the world are invited to join us online to hear leading investigators present their latest results to the NIH Intramural Research community. Lectures may be viewed live at 3:00 p.m., EST (20:00 GMT) on Wednesdays, from September through June. Live webcasts can be viewed under "Today's Events" at: <http://videocast.nih.gov/>

The current schedule of lectures is available at: <http://wals.od.nih.gov>

Upcoming Lectures:

- October 28: Robert Sapolsky, Stanford University, "Stress and Health: From Molecules to Societies"
- November 18: Dan Kastner, National Institutes of Health, "Fever, Genes, and Histories: Adventures in the Genomics of Inflammation"
- December 2, 2009: Tobias Meyer, Stanford University Medical School, "Shotgun siRNA Perturbation to Dissect Growth Factor Triggered Proliferation and Migration Signaling Systems"
- December 9: Gerard Karsenty, Columbia University Medical Center, "The Novel Physiology of Bone"

JOBS IN GOVERNMENT

“Graduate students can conduct research in our labs as part of their Ph.D. training—this has been a very productive relationship.”

—Lawrence Reiter



Sandy Miller Hays, director of the Information Office with the USDA's Agricultural Research Service (ARS), notes that work at the ARS, carried out by 2,200 scientists, touches on the lives of everyone in the United States. “It’s a very exciting place to work—we have discovery going on all the time,” she says. “It’s not just about researching a new type of soy bean—we do a lot of human nutrition research, for instance, taking an agricultural commodity and turning it into a product that everybody can use,” she says. “It’s amazing the things that we have done that have an impact on people’s everyday lives.”

Opportunities

Because some hiring government institutions are so large—the intramural program at the NIH consists of about 1,100 research groups—opportunities abound for collaborations and interactions. “Our postdoctoral fellows, in particular, have an opportunity to meet a lot of people. Just about every Nobel Prize winner in Physiology or Medicine for the past 20 years has visited the NIH,” Owens says. “Most of the members of the National Academy of Sciences who are in the biomedical research area come to talk, and we make special opportunities for postdoctoral fellows to have lunch with the big-name speakers that come in.”

Owens also points out that about 20 people at the NIH, in the office of intramural training, are assigned to determine how to enhance the careers and skills of research fellows, with most of the institutes having their own training specialists. “We offer courses in how to write grants, how to get a job, how to get CVs up to speed, how to handle interviews, all sorts of things that smaller institutions might not be able to provide,” Owens says.

EPA’s research facility at Research Triangle Park in North Carolina has been consistently ranked as one of the top places to work. The EPA forms several research collaborations with surrounding universities. “We have mechanisms whereby graduate students can conduct research in our labs as part of their Ph.D. training—this has been a very productive relationship that we have established with universities,” says Lawrence Reiter, the acting deputy assistant administrator for management. He notes that the EPA also has a federal postdoctoral program that allows recruitment for up to four years, and that these positions are posted on the USAJobs website.

At NOAA, one of the key benefits, says Sandifer, is the robust commitment to form partnerships with the external research community. “These partnerships go well beyond just providing competitive grants to academic researchers and include numerous cooperative institutes, joint programs with academia and nongovernmental organizations, involvement with students at all levels, and participation in international organizations,” he says.

Culture and Work/Life Balance

Every institution is different, but one of the benefits of a government job is that funding and resources are for the most part much more sta-

TOP REASONS FOR SEEKING A FEDERAL JOB

- **Opportunity:** The Office of Personnel Management predicts that by 2016, 40 percent of all current federal employees will have retired. Jobs are available across the US and worldwide.
- **Meaning:** Federal employees can work on issues that impact the lives of most or all of the American people.
- **Interest:** Some jobs require a unique and narrow range of skills and interests.
- **Financial:** Repayment of student loans and tuition reimbursement. Pay scales competitive and commensurate with education and experience.
- **Work force diversity:** Active encouragement for all people to seek out opportunities.
- **Work/life balance:** Job-sharing, on-site child care, competitive benefits.

TIPS FOR APPLYING FOR A FEDERAL JOB

(based on information at www.ourpublicservice.org/OPS/publications/viewcontentdetails.php?id=118)

Develop a Federal Resume

Differs from traditional resume; create and save at www.USAJobs.gov.

Fill Out a Knowledge, Skills and Abilities (KSA) Assessment

Specific to federal jobs. Requires self-ranking of skills and descriptions of job or academic experiences in first person narrative at www.makingthedifference.org

Have Patience

The process takes longer than in the private sector; appropriate to follow up with a call to the agency.

Prepare for the Interview

Treat like any other interview. Bring work examples and anything else that may be helpful.

ble and forthcoming than in academia. Miller Hays says that scientists at ARS have guaranteed funding. “So you’re not scrambling for funds every year or every two years; there’s also a tremendous amount of autonomy on the part of the scientists; you don’t have to stop and teach classes—you’re just in there doing your thing,” she says.

However, the 40-hour work week and plethora of paid federal holidays may be more myth than reality, at least at some institutions. At the NIH, Owens says that 40 hours a week is the minimum, “but many people will put in 60-hour weeks.” He admits that it can sometimes be a little hard for people who “want to have a life outside of science, but we’re trying to figure out how to balance the whole work/family issue,” he says. Nonetheless, at the NIH, a variety of extramural activities are available, including clubs for biking, judo, taekwondo, sailing, hiking, and yoga; there is even an NIH orchestra, says Phil Lenowitz, deputy director of NIH’s Office of Human Resources. “So, we cover a broad spectrum—there’s pretty much something for everybody here.”

Whatever the background, and whatever the interest, the federal government is hiring and likely has a suitable job for a qualified person.

Emma Hitt is a freelance medical and science writer residing in Marietta, Georgia.

DOI: 10.1126/science.opms.r0900081



University of Connecticut Health Center

Director, Center for Vascular Biology

The University of Connecticut School of Medicine is seeking highly qualified individuals for the position of Director of the Center for Vascular Biology. The candidate must have an excellent record of sustained NIH funding, be interested or experienced in teaching graduate students and serving as a mentor for trainees at multiple levels. The candidate should have demonstrated leadership experience and skills and recognize the importance of interpersonal relationships and team building. The candidate should be qualified for appointment at the senior rank professorial level. We are seeking an individual to complement and expand our established expertise in vascular biology and collaborate with existing cardiovascular faculty to further advance our development as a world-class research and educational program.

Candidates are invited to visit the Center web page (<http://cvb.uchc.edu>) and should apply by submitting a curriculum vita and the names of three references via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, **Bruce Liang, M.D., Chair, Vascular Biology Search Committee, Ray Neag Distinguished Professor, Director of the Pat and Jim Calhoun Cardiology Center, Chief of Cardiology, The University of Connecticut Health Center, School of Medicine, Farmington, CT 06030-3946, Email: bliang@uchc.edu**

Tenure Track Faculty Position in RNA and Stem Cells

The Department of Genetics and Developmental Biology at the University of Connecticut School of Medicine is seeking highly qualified individuals with an outstanding background in RNA biology and stem cells. Areas of interest include, but are not limited to, RNA editing, alternative splicing, large and small non-coding RNAs, RNA transport and RNA stability. Candidates who use genomic approaches to characterize the role of these processes in the maintenance of pluripotency and lineage differentiation are of particular interest. The Department is seeking an individual who will build on our established expertise in these areas and who will develop a world-class research program in RNA research with a strong stem cell component.

Applications are invited for a position at the Assistant, Associate or Full Professor level. Faculty will enjoy superb resources including a generous start-up package as well as state-of-the art core facilities for mouse transgenics and ES cell manipulation, microarrays, next-generation sequencing, flow cytometry, confocal microscopy and fluorescence imaging. The successful candidate will be expected to establish an independent and innovative research program that will attract extramural funding and to actively contribute to a rich scientific environment.

Candidates are invited to visit the departmental web page (<http://genetics.uchc.edu>) and should apply by submitting a curriculum vita and the names of three references via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, or directly to: **Marc Lalande, Ph.D., Professor and Chair, Department of Genetics and Developmental Biology, The University of Connecticut Health Center, School of Medicine, Farmington, CT 06030-3301, Email: ucsci_admin@uchc.edu**

Faculty in Cellular Nanoscience R. D. Berlin Center for Cell Analysis and Modeling

The Berlin Center for Cell Analysis and Modeling (<http://www.ccam.uchc.edu/>) at the University of Connecticut Health Center is a multi-disciplinary research center focused on development of new photonic, microscopic and computational approaches for the study of cellular systems. In the spring of 2010, we will jointly occupy a new \$50 million/117,000 sq. ft. research facility with the University's Stem Cell Institute and Department of Genetics and Developmental Biology. Strong synergies with these research groupings are anticipated. We are seeking additional faculty whose research programs elucidate nanoscale processes that control cellular function. Areas of special interest include: synthetic biology, analysis and design of cell regulatory circuits, computational cell biology, nanofabrication, nanomanipulation, micro- and nano-sensors, micro- and nano-fluidics, single molecule microscopy, non-linear optical contrast mechanisms, and new modalities for high resolution live cell imaging. Applications are welcome at any level and investigators with established funded research programs are especially encouraged to apply. Opportunities to participate in training of graduate students in cell biology, physical sciences and engineering disciplines will also be available. Salary and startup package will be commensurate with experience and level of appointment. The closing date for receipt of applications is Nov. 10, 2009.

Applicants should submit a letter of application, curriculum vitae, research plan and statement of teaching interests, and names (with address and e-mail address) of at least three references. Applications should be e-mailed in RTF or PDF format to nanocell@uchc.edu or submitted via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>

Assistant, Associate or Senior Scientist

The Geology and Geophysics Department invites applications for two full-time tenure-track positions at the Assistant, Associate or Senior Scientist level. These positions are eligible for benefits.

The first position is in the area of metamorphic petrology (fluid-rock interaction) to pursue new research directions in fluid-rock interactions and chemical fluxes between the crust, mantle and oceans, dehydration/serpentinization processes, kinetic and thermodynamic modeling of metamorphic processes and elucidating the linkages between fluid flow, alteration deformation and the chemical fluxes across subduction zones. The candidate would bridge research between geochemists and geophysicists studying the global geochemical cycle from midocean ridges to subduction zones, and the geochemical evolution of the Earth.

The second position is in the area of isotope geochemistry. The successful candidate would apply state-of-the-art technologies of isotope geochemistry to a range of problems, including timescales of geologic processes, global geochemical cycles, mantle dynamics and evolution, biogeochemical cycles, and geochemical kinetics.

Successful candidates are expected to develop and maintain their own independent externally funded research program. Opportunities exist for teaching and advising graduate students through the MIT-WHOI Joint Program in Oceanography/Applied Ocean science and Engineering, as well as for collaborating with scientists and engineers elsewhere in the institution; the Departments of Marine Chemistry and Geochemistry, Biology, Physical Oceanography, Applied Ocean Physics and Engineering, and the Marine Policy Center.

A Ph.D. is required at the time of the appointment as well as a demonstrated record of excellence in research. The level of appointment will depend on the candidate's background and experience. Women and minority applicants are particularly encouraged to apply.

Target for applications, which should include a CV, a research statement and a list of 4 references who could submit letters of recommendation is November 30th, 2009.

Please visit <http://jobs.whoi.edu> for a detailed job description and to apply online today!

WHOI is an Affirmative Action/Equal Opportunity Employer M/F/D/V. Applications are reviewed confidentially.



Woods Hole Oceanographic Institution

The Ohio State University

Departments of Plant Cellular & Molecular Biology and Molecular Genetics and the Plant Molecular Biology/Biotechnology Program

The merging Departments of Plant Cellular & Molecular Biology and Molecular Genetics at The Ohio State University, in conjunction with the interdisciplinary Plant Molecular Biology/Biotechnology (PMBB) program, invite applications for a full-time, tenure-track faculty position. Appointment at the Assistant Professor level is preferred, but outstanding candidates at senior levels will also be considered. This position is part of a new campus-wide Targeted Investment in Excellence initiative awarded to the PMBB Program in Translational Plant Sciences. Successful applicants are expected to develop an outstanding research program and to participate in teaching at the undergraduate and graduate levels. Applicants with expertise in any areas of plant molecular, cellular, biochemical, developmental, genomics and systems biology are encouraged to apply. Research programs addressing fundamental problems of bioenergy, bioproducts, carbon sequestration or plant-microbe interactions are of particular interest.

Plant science is an expanding area of research at the university (see other plant-related positions at <http://hcs.osu.edu/index.php/useful-links/news-stories/front-page/143-assistant-professor-of-plant-biochemistry> and <http://orps.osu.edu/>). Departmental faculty members also participate in numerous campus-wide programs, centers and focus groups that include PMBB, the Plant Biotechnology Center, the Molecular Plant-Microbe Interaction Group, the Cell Biology Group, the Developmental Genetics Group, the Center for RNA Biology, the Institute for Energy and the Environment, the Mathematical Biosciences Institute and the Arabidopsis Biological Resource Center.

The Ohio State University is the flagship institution of the higher education system in Ohio. It is located in Columbus, the state capital, which is a vibrant large city that has been continuously ranked as one of the country's best places to live and work. Further information about the departments, PMBB, the university and Columbus can be obtained at <http://www.biosci.ohio-state.edu/~plantbio/plantbio.html> (Plant Cellular & Molecular Biology), <http://www.osumolgen.org> (Molecular Genetics) and <http://www.ag.ohio-state.edu/~pmbb/> (PMBB). Flexible work options are available.



Candidates with a Ph.D. and suitable postdoctoral experience should submit applications including curriculum vitae, a 3 page or less research plan and a brief description of teaching experience and interests. Candidates for consideration at the Assistant Professor level should also arrange to have at least 3 reference letters submitted. Electronic applications and reference letters to pbsearch@biosci.osu.edu are preferred, but paper copies may also be sent to Search Committee Chair, Dept. of Plant Cellular and Molecular Biology, Ohio State University, 500 Aronoff Laboratory, 318 West 12th Ave., Columbus, OH 43210-1242. Review of applications will begin November 15, 2009 and continue until a suitable candidate is identified.

To build a diverse workforce Ohio State encourages applications from individuals with disabilities, minorities, veterans, and women. EEO/AA Employer. Ohio State is an NSF Advance institution.

IDA Science & Technology Policy Institute: Now Hiring

The Institute for Defense Analyses Science and Technology Policy Institute [STPI] seeks talented individuals to lead studies in three broad areas: (1) life sciences and biomedical research; (2) energy and the environment, and (3) program evaluation, portfolio analysis, and scientometrics.

STPI, a Federally Funded Research and Development Center operated by the Institute for Defense Analyses, provides analytical support to the Office of Science and Technology Policy [OSTP], the National Institutes of Health, and other federal agencies on science, technology, and innovation policy issues. Candidates must be knowledgeable, practiced, dynamic scientists, engineers, or social scientists with significant policy understanding and comprehension. We are seeking individuals who will conduct and lead policy studies in each of the three areas. Requirements for each area are:

(1) Life Sciences: Ph.D. or M.D. staff to conduct and lead policy analysis studies in the areas of life sciences, healthcare and the conduct of clinical and translational research.

(2) Energy and the Environment: Ph.D. to undertake and lead studies on energy-related technologies, policies, and social and behavioral issues associated with the adoption of new energy technologies.

(3) Evaluation and Scientometric: Ph.D. and accomplished policy analyst experience with a background in public administration, program evaluation, or organizational behavior.

The ideal candidate will have research experience in an academic or industry environment as well as experience with some aspect of R&D management/administration or S&T policy. Experience levels desired are senior staff with 10-15 years experience. The successful applicant must have a demonstrated ability to work within a team of S&T policy staff and to express ideas clearly in spoken and written format. STPI is located in downtown Washington DC, just a few blocks from OSTP and the White House.

IDA offers competitive salary, an excellent benefits package and a superior professional working environment. The selected individual will be subject to a security investigation and must meet the requirements for access to classified information. U.S. citizenship is required. IDA is proud to be an equal opportunity employer. Interested candidates should apply online at www.ida.org - keyword STPI.



INSTITUTE FOR DEFENSE ANALYSES

PICTURE YOURSELF AS A AAAS SCIENCE & TECHNOLOGY POLICY FELLOW

Make a Difference.

Help give science a greater voice in Washington, DC! Since 1973, AAAS Fellows have applied their skills to federal decision-making processes that affect people in the U.S. and around the world, while learning first-hand about the government and policymaking.

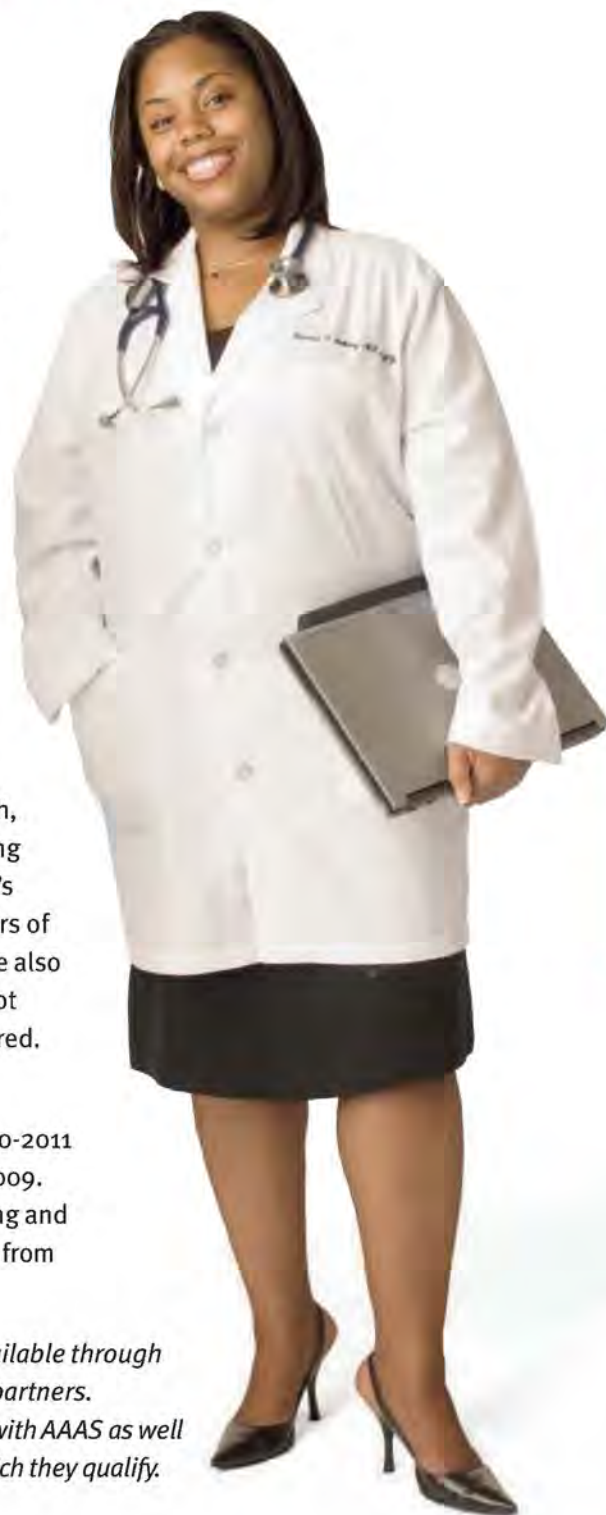
Join the Network.

Year-long fellowships are available in the U.S. Congress and federal agencies. Applicants must hold a PhD or equivalent doctoral-level degree in any behavioral/ social, biological, computational/ mathematical, earth, medical/health, or physical science, or any engineering discipline. Individuals with a master's degree in engineering and three years of post-degree professional experience also may apply. Federal employees are not eligible and U.S. citizenship is required.

Apply.

The application deadline for the 2010-2011 AAAS Fellowships is 15 December 2009. Fellowships are awarded in the spring and begin in September. Stipends range from \$73,000 to \$95,000.

Note: Additional fellowships are available through approximately 30 scientific society partners. Individuals are encouraged to apply with AAAS as well as with any scientific societies for which they qualify.



*Enhancing Public Policy,
Advancing Science Careers*

Renaisha S. Anthony, MD

University of Chicago,
Pritzker School of Medicine

2007-08 AAAS Fellow at
the National Institutes of
Health, National Institute
of Child Health and Human
Development

Now on the faculty at George
Washington University, School
of Public Health, Department
of Prevention and Community
Health

Full details at: **fellowships.aaas.org**


ADVANCING SCIENCE. SERVING SOCIETY



Xi'an Jiaotong University Health Science Center(XJTUHS) is recognized nationally and internationally for its outstanding achievements in medical research and education. Based in Xi'an, a very famous city with a long history, **XJTUHS** consists of a Medical College and 4 big affiliated teaching hospitals, mainly focuses on medical education and basic and applied medical sciences research. **XJTUHS** invites applications for full-time tenure-track faculty positions at the level of professor or associate professor. We are especially looking for individuals with a strong background in biomedical sciences and experience addressing challenging biological questions in areas such as stem cells and development, neurological disorders, metabolic diseases, DNA instability and cancer, epigenetics, genomics, proteomics, bioinformatics and biotechnology, physiology and pathophysiology, pharmacy, pharmacology, anatomy and embryology, immunology and pathogen biology, pathology, medical experimental zoology, public health, and other any researches related to medical research. We also looking for good physicians and surgeons with high level education and good experiences. Successful candidates should demonstrate a record of ongoing scholarly activity and strong potential for obtaining extramural research funding. Candidates should have a PhD or MD degree with substantial relevant postdoctoral experience and a proven record of research excellence. An interest in teaching is also important. We have an active mentorship program for tenure-track faculty, which provides ample opportunities for the development of independent and collaborative research programs within our center and across the university, and strives to provide a stimulating and supportive environment for medical science research. Talented, enthusiastic researchers working on fundamental biological processes or pathogenesis of human diseases are encouraged to apply. Successful candidates will enjoy newly renovated space, highly collegial and interactive environment, and generous startup funding from the Guanghua Hundred Talents Program. Salaries will be commensurate with experiences and qualifications. For more information please visit: <http://yxzx.xjtu.edu.cn>

Applications will be reviewed quarterly until the positions are filled. Please submit CV, statement of current research summary (up to 2 pages) and future research plan (up to 2 pages), and names of three professional references to:

Bin Wu, assistant of Director,
Office of Xi'an Jiaotong University Health Science Center;
Tel: 86-29-82656852;
Email: xjtuhsc@163.com



Worcester Polytechnic Institute

Head of Chemistry and Biochemistry And Endowed Professorship

WPI invites applications and nominations for Head of the Department of Chemistry and Biochemistry who will also hold an Endowed Professorship. The Department is integral to a major life science research initiative at WPI, supported by a new, state-of-the-art research facility that opened in the spring of 2007. WPI seeks a Department Head with a clear and creative vision for building and sustaining ambitious departmental research programs during a period of growth. This vision will include enhancing the department's role in the natural sciences, and the successful candidate will be expected to oversee a vigorous faculty recruitment program. The Department Head will have an earned doctorate in chemistry or biochemistry, an international reputation in research, and a distinguished record of publication and funding in an area of chemistry or biochemistry. She or he must demonstrate outstanding leadership and mentoring abilities as well as a commitment to high quality teaching at both the undergraduate and graduate levels.

The Department of Chemistry and Biochemistry at WPI offers undergraduate and graduate (MS, Ph.D.) degrees in chemistry and biochemistry. WPI is on the Chronicle of Higher Education's 2009 list of "Great Colleges to Work For." Further information about WPI and the department can be accessed at <http://www.wpi.edu>

The application should consist of a detailed curriculum vita, a letter of intent that describes professional interests (research, teaching, and administrative), and a vision statement for the future development of the department. Applications and nominations should be sent to cbheadsearch@wpi.edu. Further enquiries can be directed to the chair of the search committee, Dr. Richard Sisson (sisson@wpi.edu) or the Head of Chemistry and Biochemistry, Dr. Kristin Wobbe (kwobbe@wpi.edu).

To enrich education through diversity,
WPI is an affirmative action, equal opportunity employer.
— A member of the Colleges of Worcester Consortium. —

Senior Vice Provost for Research Administration & Graduate Education

Temple University is the 27th-largest university in the United States and one of the nation's leading centers of professional education. Temple University's Graduate School offers 121 master's programs, 56 doctoral programs, and seven first-professional degree programs and is among the nation's largest educators in the fields of medicine, dentistry, pharmacy, podiatry, and law. Temple University is located in Philadelphia, Pennsylvania, a vibrant city with a diverse student population.

For information about this position, please visit us at <http://www.temple.edu/provost/svprage>



Temple University is an Equal Employment Opportunity/Affirmative Action Employer. Nominations and applications from women and minorities are encouraged.

Temple University invites applications and nominations for the position of Senior Vice Provost for Research Administration & Graduate Education.

The Senior Vice Provost for Research Administration & Graduate Education reports to the Executive Vice President for Academic Affairs/Provost and works closely with the deans, faculty, and other leaders to set a strategic direction in line with the university's Academic Strategic Compass. Specific responsibilities include:

- Providing executive leadership in support of our research administration and graduate education programs.
- Developing a strategic focus that contributes to the overall achievement of the University as a premier research institution. This will include increasing the level of sponsored research throughout the University and promoting a climate that encourages excellence in research and creative expression.
- Cultivating relationships with external funding agencies and providing administrative and technical leadership to support the enhancement of research and economic development.
- Providing leadership for the full range of issues related to the university's research centers and institutes, research infrastructure, core research, and service programs.

Temple University seeks candidates who demonstrate scholarly and academic credentials. Professional recognition as a scholar among peers is essential. Candidates should have extensive senior-level administrative experience in a higher education environment.

Screening will begin immediately and continue until an appointment is made. All communications will be treated confidentially. Nominations, inquiries, and applications (including a cover letter, curriculum vitae, and the names of five references) should be directed electronically in confidence to: **Harry A. Young, SPHR, Associate Vice President, Human Resource Operations, Temple University, 1601 North Broad Street, Philadelphia, PA 19122.** svprage@temple.edu

Stanford Institute for Stem Cell Biology and Regenerative Medicine (ISCBRM) Tenure-line Faculty Member

The Stanford Institute for Stem Cell Biology and Regenerative Medicine (ISCBRM) is holding an open search for a tenure-line faculty member in the areas of adult stem cell biology and its relation to organ or tissue function, cancer stem cell biology including targeted immune therapies to cancer stem cells, embryonic stem cell biology, and mechanisms of somatic cell reprogramming to produce human pluripotent stem cell lines. This position is ideal for a stem cell scientist in the early or mid-stage of his/her career but who is already established as an outstanding investigator.

The successful candidate will have an outstanding record of research and a strong interest in helping to translate these discoveries into pre-clinical research and potential therapies. All appointments to the ISCBRM also have primary appointments in departments at Stanford University. Interested candidates need to indicate preferences for potential department affiliation. The appointee, however, will work on location in the ISCBRM laboratories and will participate in ISCBRM research and teaching activities. While excellence in teaching is an important criterion, the appointment will be based primarily on research accomplishments and the promise of future research and translational medicine advances. Salary will be commensurate with the level of employment, relevant experience, and accomplishments.

Please address and mail letters of interest, along with full curriculum vitae and the names and addresses of three references to:

Susan Prohaska, Ph.D., ISCBRM Program Officer
or **Irving L. Weissman, M.D.**

**Director, Institute for Stem Cell Biology
and Regenerative Medicine**
ISCBRMFacultysearch@stanford.edu

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes applications from, and nominations of, women and members of minority groups, as well as others who would bring additional dimensions to the university's research, teaching, and clinical missions.



Northeastern University

**Assistant/Associate/Full Professor
Movement Neuroscience**

The Departments of Biology and Physical Therapy at Northeastern University are seeking interdisciplinary applicants in **experimental or computational Movement Neuroscience**. All ranks will be considered. Individuals working on neurophysiological, biomechanical and executive functions in the control of movement, rehabilitation and restoration of function, development, sensorimotor integration, muscle function and other areas related to motor control are encouraged to apply. We are interested in individuals with a variety of research methods ranging from behavioral, neurophysiological, biomechanical, electrophysiological, engineering and imaging techniques. Applicants will be expected to support a strong research program and contribute to teaching in the area of neurobiology and biomechanics of movement at both the graduate and undergraduate levels. Successful applicants are expected to maintain an independent research program of distinction, as well as effective collaborations with other faculty at Northeastern University and institutions.

Qualifications include a PhD, a strong record of scientific achievements and extramural funding commensurate with the level of the appointment, and a willingness to contribute to undergraduate and graduate teaching.

Start date: Fall 2010.

To apply, visit the website <http://www.northeastern.edu/cas/> and click on 'Faculty Positions'. For questions about the search, call: **Dagmar Sternad, 617-373-5093**. Review of applications will begin **January 15, 2010** and continue until the position is filled.

Northeastern University is an Equal Opportunity, Affirmative Action Educational Institution and Employer, Title IX University, and NSF funded ADVANCE Institutional Transformation site. Women and minority candidates are encouraged to apply.



TEMASEK RESEARCH FELLOWSHIP

The Temasek Research Fellowship (TRF) is a prestigious scheme aimed at recruiting outstanding young researchers at the post-doctoral level to undertake research as Principal Investigators and lead teams to undertake defence-related research in the Nanyang Technological University (NTU) or National University of Singapore (NUS) in Singapore.

The TRF is a 3-year Fellowship with an option to extend up to 3 years. The Temasek Research Fellow (TRF-RF) may be offered a faculty appointment at the end of their term.

In addition to an attractive remuneration package that will commensurate with qualification and experience, the TRF-RF will be provided a research grant to pursue his/her research at Temasek Laboratories at NTU or NUS or other research entities at the respective Universities.

For more information and application procedure, please visit:
NTU - <http://www.ntu.edu.sg/trf>
NUS - <http://www.nus.edu.sg/dpr/funding/fellowship.htm>

Closing date: 22 December 2009

Short-listed candidates will be invited for an interview and scientific presentation expected to be held in March 2010



LUDWIG-
MAXIMILIANS-
UNIVERSITÄT
MÜNCHEN

FACULTY OF PHYSICS

To strengthen and widen the scope of the nanosciences at the Ludwig-Maximilians-Universität München (LMU), the **Faculty of Physics and cluster of excellence "Nanosystems Initiative Munich" (NIM)** invite applications for a

**Professorship (W2)
for Theoretical Physics in the area
of Functional Nanosystems
(tenure track)**

The tenure track position is initially for six years and can be converted to tenure pending a positive evaluation after a minimum of three years. In exceptional cases a tenured position can be offered directly.

Possible fields of research include all questions relevant for understanding, designing and manipulating functional nanosystems. Preferred focus areas are electronic, magnetic, photonic, atomic and mechanical nanosystems.

Participation in the interdisciplinary activities of the "Center for NanoScience" (CeNS), the LMU *innovativ* focus area "Functional Nanosystems", the "Arnold Sommerfeld Center for Theoretical Physics" (ASC), as well as in local collaborative research programs is possible. For additional information on ongoing activities see <http://www.nano-initiative-munich.de> and www.cens.de.

Candidates are expected to conduct an independent research program that complements existing research efforts and to participate in the teaching program.

Prerequisites for employment are a university grade, pedagogical suitability, Ph.D. or doctoral degree and additional scientific qualification which can be proved by a habilitation or equivalent achievements.

In general the age of 52 should not be exceeded at the time of appointment, though exceptions are possible.

The advancement of women in science is an integral part of the university's policy. Women, therefore, are especially encouraged to apply.

Persons with disabilities will be given preference over other applicants with comparable qualifications.

The Ludwig-Maximilians-Universität München offers support for couples of double career.

Applications including a curriculum vitae, academic records, a list of publications, as well as a two-page summary of planned research activities should reach the **Dekan der Fakultät für Physik der Ludwig-Maximilians-Universität München, Schellingstr. 4, 80799 München, Germany**, not later than **December 1, 2009**.



UNIVERSITY OF UTAH SCHOOL OF MEDICINE

TENURE TRACK FACULTY POSITION in NEUROSCIENCE

The Dept of Neurobiology and Anatomy at the University of Utah (<http://www.neuro.utah.edu/>) is seeking an outstanding scientist for a tenure track faculty position at the Assistant Professor level. We are interested in candidates who are pursuing fundamental problems in neuroscience. Areas of interest include but are not limited to neural circuitry and plasticity, behavioral genetics, and aging, regeneration and repair.

Individuals holding Ph.D. and/or M.D., or equivalent degrees, with two or more years of postdoctoral experience and using innovative combinations of molecular, genetic, and cellular approaches are encouraged to apply. Applicants should demonstrate excellence in research and strong potential for securing and sustaining independent and collaborative extramural funding.

The University of Utah offers excellent resources to support new faculty, including competitive salary and start-up support, a highly collegial research environment, core facilities and strong interdepartmental graduate training programs. A successful applicant will be expected to develop an innovative, independent research program, and to share our commitment to excellence in graduate and medical education.

Only electronic applications will be accepted. Please submit a single PDF document including: (1) cover letter, (2) curriculum vitae, (3) research statement (4) one recent publication. Email the application to: facsearch@neuro.utah.edu. Three letters of reference should be sent independently to: facsearch@neuro.utah.edu. For full consideration, applications should be received by **December 1, 2009**.

The University of Utah is an Affirmative Action/Equal Opportunity Employer and does not discriminate based upon race, national origin, color, religion, sex, age, sexual orientation, gender identity/expression, disability, or status as a Protected Veteran. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities. To inquire about the University's nondiscrimination policy or to request disability accommodation, please contact: Director, Office of Equal Opportunity and Affirmative Action, 201 S. Presidents Circle, Rm 135, (801) 581-8365.

The University of Utah values candidates who have experience working in settings with students from diverse backgrounds, and possess a demonstrated commitment to improving access to higher education for historically underrepresented students.



DONALD DANFORTH PLANT SCIENCE CENTER

Faculty Position in Renewable Fuels

As part of the continuing expansion of the Enterprise Rent-A-Car Institute for Renewable Fuels at the Donald Danforth Plant Science Center, a faculty position at any level is available for a scientist with research interests in the area of renewable fuels using plant or algal systems. Investigators addressing fundamental questions related to the bioenergetics, molecular biology, or biochemistry of biomass, hydrocarbon or renewable feedstock production are especially encouraged to apply. Postdoctoral experience and a record of excellence in research are required. The successful candidate is expected to establish a strong, creative and collaborative research program.

Please submit a curriculum vitae, a statement of research plans and three reference letters to: Ms. Billie Broeker, Director of Human Resources, RE: ERAC, Donald Danforth Plant Science Center, 975 N. Warson Rd, St Louis, MO 63132. Or by email to: bbroeker@danforthcenter.org with ERAC in the subject line. Review of applications will begin **November 30, 2009**. For more information please visit <http://www.danforthcenter.org>.

EOE/AA EMPLOYER/M/F/D/V



Institute for Structural and Molecular Biology (ISMB)

At University College London – Birkbeck College London

Lectureship in "Reconstitution of Cell Biological Systems"

The Institute for Structural and Molecular Biology (ISMB) is seeking applications to fill a lectureship in the Reconstitution of Cell Biological Systems.

We want to recruit outstanding individuals who are capable of developing world-class, interdisciplinary research in Cellular and Molecular biology. The successful applicant will undertake research on the function of complex cellular systems (for example endocytic machineries, signalling complexes, cell division, nucleopore, etc...). We are especially interested in expertise with systems that are amenable to *in vitro* reconstitution, and functional analysis using a range of molecular, cellular, biochemical and biophysical approaches. The successful applicant will also be expected to make an appropriate contribution to the affiliated departments portfolios of graduate and undergraduate teaching.

The salary range that is offered for this post will be within the University Lecturer (G8: £39,313 - £46,403 per annum inc. LW) range according to experience. The job description and person specification can be found at www.ismb.lon.ac.uk/JD1CB.html and at the respective departmental web sites (<http://www.smb.ucl.ac.uk/> and <http://www.cyst.bbkc.ac.uk/>).

To apply, please visit the UCL Vacancies website and follow the on-line application process:

<http://www.ucl.ac.uk/hr/vacancies/adverts/job-list.html>. CVs should contain an account of applicant's current research activities together with a plan of future research intentions. The Head of the ISMB, the UCL Research Department of Structural and Molecular Biology and of the Birkbeck Department of Biological Sciences is Professor Gabriel Waksman. He can be contacted informally for general information (email g.waksman@ucl.ac.uk or g.waksman@bbk.ac.uk).

Deadline for applications is 30th November 2009.

UCL Taking Action For Equality.



Faculty Positions in Coastal Hydrogeology and Geochemistry Skidaway Institute of Oceanography

The Skidaway Institute of Oceanography (SkIO) invites applications for two faculty positions available 1 July 2010. These scientists will complement SkIO's existing programs in marine and coastal geology, sediment and stable isotope geochemistry, coastal physics, microbial ecology and environmental chemistry. Applicants are encouraged to learn more about the Institute and its programs at www.skio.usg.edu.

Coastal Hydrogeology: SkIO seeks candidates with demonstrated interest in interdisciplinary research on material and water exchange between surface waters, surficial aquifers and/or intertidal wetlands, the role of these hydrologic systems in the transfer and fate of carbon, nutrients and/or other materials in the coastal zone and applications of models to these processes.

Marine Geochemistry: Candidates with interests in either inorganic or organic geochemistry are encouraged to apply. While individuals from all sub-disciplines of geochemistry may apply, we particularly welcome applicants with interests in carbon cycling within coastal marine waters. A history of collaboration or leadership in interdisciplinary research is an advantage.

The appointments will be made at either the Assistant or Associate Professor level, depending upon qualifications. Candidates for both positions should demonstrate strong evidence of (or potential for) the ability to conduct an active, extramurally funded research program. While SkIO is primarily a research institution, there are opportunities for interactions with local and regional academic institutions. Some level of participation in joint educational programs is expected. Send CV, statement of research interests and teaching experience and contact information for at least three references to resumes@skio.usg.edu. Please attach required documents in PDF format. Include either Hydrogeologist search or Geochemist search in the subject line. Electronic applications are strongly encouraged, but paper applications may be sent to Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA, 31411. Review of applications will begin on **November 30, 2009** and will continue until the positions are filled.

EOE/AA Employer.

EXECUTIVE DIRECTOR

for the International Barcode of Life (iBOL) Project



Background: Biodiversity on our planet is imperiled and efforts to reverse this situation are compromised by difficulties in mapping species distributions. DNA barcoding, the use of sequence diversity in short, standardized gene regions to create a digital identification system for life, will empower biodiversity science by enabling automated species identifications. Work over the past 5 years has delivered barcode records for 50K species and has provoked plans for iBOL, a genomics project which will both massively expand the DNA barcode reference library and develop the technologies required to read it. An administrative structure has now been established to support this research program which is the largest initiative ever undertaken in biodiversity genomics (www.iBOLProject.org). The International Barcode of Life (iBOL) consortium has been established as a stand-alone, not-for-profit corporation, overseen by an international Board of Directors representing participating funders. By 2015, iBOL consortium members will have gathered DNA barcode records from 5M specimens of 500K species, producing an identification system for species that are regularly encountered by humanity and a solid foundation for subsequent advance toward the DNA barcode reference library for all eukaryotes. The impacts on biodiversity science and its applications throughout society will be transformative. The Executive Directorship of iBOL is an extraordinary opportunity for someone with administrative verve, imagination and a strong sense of mission. More than 25 nations are participating and major commitments have been made toward the target operating budget of \$150M for this first phase of iBOL (2010-2015).

Position Description: The iBOL secretariat is based at the Biodiversity Institute of Ontario, a research unit of the University of Guelph in Ontario. The secretariat is responsible for co-ordination of the overall iBOL research program and for the assembly of reports to the Board of Directors and the participating funding agencies it represents. Reporting to the Scientific Director (SD) of iBOL, the Executive Director will oversee the secretariat and work closely with the SD to achieve consortium goals. Aided by a core staff, the Executive Director will have responsibility for administration and operations of the iBOL consortium. These duties will include, either directly or in a coordinating role, the cultivation of iBOL consortium partners; consortium governance and accountability; internal and external reporting and communication. The Executive Director will work with other international biodiversity, genomics and scientific organizations which represent key opportunities for cooperation or collaboration. It is anticipated that the position will require significant domestic and international travel.

Required Qualifications and Skills: PhD in the life sciences or equivalent combination of experience and education; MBA or equivalent combination of experience and education; strong organizational and management record with 10+ years of experience, preferably including significant roles in large-scale research projects and/or consortia, supervisory responsibilities, and success with managing international partnerships and/or collaborations; excellent written and oral communication skills; strong interpersonal and leadership record. The Executive Director should have the capacity to stimulate enthusiasm for the project and the energy to help build a transformative project on a dynamic and complex funding and scientific landscape.

Additional Information: Further information on the International Barcode of Life project is available at www.iBOLProject.org. Submit your resume (including the names of three potential referees) and a cover letter in confidence to EDsearch@iBOLproject.org by **November 15, 2009**. References will only be requested after individuals placed on the shortlist have authorized contact. Start date is flexible, but April 1, 2010 is the target.

The Division of Basic Pharmaceutical Sciences, Florida A&M University College of Pharmacy and Pharmaceutical Sciences invites applications for two full-time 12-month positions in the areas of **Molecular Biology and Pharmaceutics**.

Molecular Biology Position: The incumbent will have a PhD degree in the area of molecular biology. Areas of interest include but not limited to the identification of the molecular mechanisms of drug action and understanding of the molecular basis of toxicity. This is initially a non-tenure track position but it has the potential to become a tenure-track position based on performance.

Pharmaceutics Position: Individuals with experience and vision to develop research in the areas of drug delivery, nanotechnology and/or biopharmaceutics are encouraged to apply. A PhD degree in the discipline of pharmaceutics, physical pharmacy and/or biopharmaceutics is required.

The successful candidates would be expected to teach at the PharmD and graduate levels. The positions are at the Assistant/Associate Professor levels depending on qualification and experience. Review of applications will begin immediately and will continue until the positions are filled. Applicants should send a letter of interest, curriculum vitae, description of research interests and three letters of reference to: **Seth Y. Ablordepey, PhD., Chair, Division of Pharmaceutical Sciences, College of Pharmacy, Florida A&M University, Rm 201 Dyson Pharmacy Building, Tallahassee, FL 32307.** Electronic submissions are welcomed. Send email with "Basic Pharmaceutical Sciences Application" as subject and addressed to seth.ablordepey@famuedu.

GRADUATE PROGRAM



Wellcome Trust Sanger Institute Four-Year PhD Studentships

Registered at the University of Cambridge

The Wellcome Trust Sanger Institute is internationally recognized as one of the world's leading genome centres, and our programmes are at the forefront of experimental and computational genome research. These research programmes emphasise investigation of human genetic variation in health, cancer and other diseases, experimental genetic analysis of gene function in a variety of model organisms, biological studies of human and animal pathogens, and computational interpretation of genomic data.

Progress in biology is increasingly dependent on genomics, thus training in this area is not only rewarding, but will offer many opportunities in the future. Our graduate training programme is well established and has substantial support from the Wellcome Trust. We welcome applications from students who wish to join this exciting phase of our research as we move from genome sequence to genome function.

Highly competitive fully funded four-year studentships (tuition fees and stipend) will be available from October 2010 in the areas of genetics, functional genomics and computational biology. Studentships are open to UK and international students. For a full list of faculty research interests please see <http://www.sanger.ac.uk/Teams/faculty>.

For more information about our graduate programme and details on how to apply please visit our website <http://www.sanger.ac.uk/careers/phd/>.

Application closing date: 12th December 2009.

Please note that applying to our PhD programme is free.

Wellcome Trust Sanger Institute Clinical PhD Fellowships

We also provide funding for two Clinical Research Training Fellowships each year as part of the Cambridge Wellcome Trust PhD Programme for Clinicians. For more information please see <http://www.cimr.cam.ac.uk/study/wtclinicians.html>.

**Research Leader
Emerging Pathogen Discovery/Molecular Virology**

The Department of Infectious Diseases and Pathology at the University of Florida College of Veterinary Medicine is seeking a tenure-track research leader at the rank of Associate or Full Professor to join us in building a team of infectious disease researchers with expertise in clinical virology and epidemiology. The Department (<http://www.vetmed.ufl.edu/college/departments/patho/>) has strong ties to the UF Emerging Pathogens Institute (<http://epi.ufl.edu>), which coordinates a multidisciplinary research program in infectious diseases across eight UF Colleges. The primary responsibility of the incumbent will be to:

- maintain a competitive extramurally-funded research program utilizing tools of molecular virology in virus discovery research and the study of diseases of veterinary and/or public health interest;
- provide expertise in emerging diseases or foreign animal diseases; and
- assist in recruiting additional faculty to complete the research team designed to create synergies and collaborative research opportunities.

Applicants must have a DVM/PhD, MD/PhD or PhD and a record of NIH R01 or equivalent extramural funding as an independent investigator. Excellent research facilities for this position are available within a dynamic and expanding Health Science Center (<http://www.health.ufl.edu/>), the country's only academic health center with six health-related colleges located on a single, contiguous campus. Applicants should submit a letter outlining professional goals, curriculum vitae, and list of three professional referees to:

Dr. William L. Castleman, Search Committee Chair, Department of Infectious Diseases and Pathology, College of Veterinary Medicine, P.O. Box 110880, University of Florida, Gainesville FL 32611-0880; e-mail castlemanw@vetmed.ufl.edu; fax: 352-392-9704. Review of applications will begin December 7, 2009 and will continue until the position is filled.

The University of Florida is an equal opportunity employer.



Professor of Comparative Medicine

Texas Tech University (TTU) and the Texas Tech University Health Sciences Center (HSC) have collaborated to form a new Institute for Comparative and Experimental Medicine (ICEM). This new Institute will support the One Health National Initiative to study and provide solutions to human and animal health concerns. The institute will recruit a number of new faculty, assign and remodel new space suited to this line of research, and provide competitive start up funds in areas of comparative medicine including, but not limited to: • Infectious disease, vaccine development, immunology, or allergy • Inflammation • Bone health • Cardiovascular health • Diabetes and Obesity • Neuroscience • Imaging and Image Processing • Addiction • Cancer • Biomedical Engineering • Genomics and Metabolomics • Bioinformatics, Modeling and Advanced Biomedical Computing • Basic, translational and clinical studies

Collaboration with existing and newly hired ICEM faculty members in both computational and experimental research and interaction in a team environment are expected. Funds are available to remodel and equip shell space in our Experimental Sciences Building (ESB) for faculty who will be affiliated with TTU. Opportunities exist to collaborate on human clinical studies in the Texas Tech System. New faculty hires will have current competitive grant funding and a distinguished record of research productivity. Graduate teaching and advising are expected in areas of interest to the faculty. Service to the institutions and the profession are important aspects of successful faculty candidates. The primary appointment may be in any academic department at either institution. The level of academic appointment is open to all ranks though there is a preference for positions at the Associate/Full Professor level.

Applicants must submit a letter of interest expressing their vision for research contributions and interactions, graduate education goals, a complete resume/CV, the names of five references, and start-up requirements to: **Institute for Comparative and Experimental Medicine, Texas Tech University, Experimental Sciences Building, Lubbock, TX 79409-3132**. For more information visit <http://www.icem.ttu.edu>. Applications should be submitted to icem@ttu.edu. These positions shall remain open until filled.

Texas Tech University System is an Equal Opportunity Institution.

**STONY BROOK
UNIVERSITY
MEDICAL CENTER**

Stony Brook University Medical Center, the only academic medical center on Long Island and the only tertiary care facility in Suffolk County, offers exciting opportunities to join our staff.

Pathology Associate or Full Professor

Stony Brook University Medical Center's Department of Pathology invites applications from outstanding, interactive, and creative scientists who are qualified for appointment at the level of a tenured associate or full professor and have proven their ability to develop and apply novel concepts in basic or translational cancer research or stem cell research.

The successful candidate will have a solid track record of past funding at the national level and is expected to lead a strong, independent research program, attract external funding, and actively seek collaborations both within the department, university, and with our closely affiliated partner institutions: Cold Spring Harbor Laboratory and Brookhaven National Laboratory.

For a full position description, application procedures, or to apply online, visit: www.stony-brook.edu/jobs (JOBS Reference #: F-6035-09-09)

Equal Opportunity/Affirmative Action Employer: Women, people of color, individuals with disabilities, and veterans are encouraged to apply. Call (631) 444-4700 for a disability-related accommodation.



Cold Spring Harbor Laboratory is seeking a highly motivated applicant in the area of

**DEVELOPMENTAL
NEUROSCIENCE**

The primary research focus of our highly collaborative faculty is neural circuits, including both how they function normally & how their disruption can lead to neuropsychiatric disorders. CSHL faculty approach questions at the genetic, molecular, developmental, systems, behavioral, computational & theoretical levels.

The successful candidate will have an outstanding record of research achievement & the ability to attract significant extramural research support. Of particular interest to this search are candidates using modern approaches to study neural circuit development & plasticity in rodent models. Applicants at the Assistant Professor & Associate Professor level will be considered.

Cold Spring Harbor Laboratory is a world-renowned research & educational institution with programs in cancer, neuroscience, plant biology, genomics & bioinformatics. The Laboratory is recognized internationally for its excellence in research & educational activities.

Review of applications will begin November 1, 2009 & will continue until positions are filled.

A cover letter, CV, description of research accomplishments & future research plans, & three letters of reference should be sent via email (PDF) with the subject line DEVELOPMENTAL NEUROSCIENCE SEARCH to the following address: devneuro09@csih.edu

CSHL is an Equal Opportunity/Affirmative Action Employer.
For more information: www.cshl.edu



**Karolinska
Institutet**

Karolinska Institutet invites applications for a Postdoc or Senior Scientist in Mouse Molecular Genetics / Molecular and Systems Biology

A position is available immediately in the laboratory headed by Prof. Jussi Taipale (jussi.taipale@ki.se) at the Department of Biosciences and Nutrition, Karolinska Institutet. Current lines of work include genome-wide RNAi screening for regulators of signaling pathways and the cell cycle, and analysis of the molecular mechanisms of growth control in cultured cells and in mouse genetic models. Research in the laboratory combines in silico analyses with mouse molecular genetics to study function and regulation of expression of key cell cycle genes (see for example Hallikas et al. Cell 124:47-59, 2006; Björklund et al., Nature 439:1009-1013; Varjosalo et al., Cell 133:537-548, 2008; Tuupanen et al., Nature Genetics 41:885-890, 2009). The project is funded in part by a European Research Council (ERC) Advanced Grant for Frontier Research.

Qualifications:

Applicants with strong backgrounds in mouse molecular genetics who are interested in working in an interdisciplinary environment are particularly encouraged to apply.

Please send your application, CV and three letters of reference marked with reference number 5209/2009, to reach us by no later than December 15 2009 to Karolinska Institutet, Department of Biosciences and Nutrition, att: Vivian Hildebrand, SE-141 57 Huddinge, Sweden. Please go to www.ki.se for more information.

The Graduate School of Nanoscience and Technology at KAIST invites applications for full-time, tenure-track or tenured faculty positions.

The Graduate School of Nanoscience and Technology is a newly established graduate program in the College of Natural Sciences at KAIST. Recently awarded the WCU (World Class University) project from Ministry of Education, Science, and Technology, we are looking for scientists who can create knowledge and develop high-impact technology in the interdisciplinary area of nanoscience and technology where physics, chemistry, biology, materials science, and engineering come together. Applicants must have a Ph.D., and some post-doctoral research experience is desirable. However, candidates from all fields, nationality, and ethnicity will be considered equally, and are encouraged to apply. The position is for a junior level professor, but senior level appointments are possible depending on qualifications.

Interested candidate should submit official application form (available at http://www.kaist.edu/english/02_faculty/01_recruit_02.php?pt=22) including publication list, research plan and teaching plan and at least three letters of recommendation to Prof. Jung H. Shin, Head, Graduate School of Nanoscience and Technology, KAIST, Daejeon, 305-701, Korea.

Applications completed by **Jan 1st 2010** will be given full consideration, but the search will continue until the positions are filled. If you have any further inquiry, please contact Program head at jhs@kaist.ac.kr.

HUMBOLDT-UNIVERSITÄT ZU BERLIN



Within the framework of the newly founded Cluster of Excellence (www.neurocure.de) the Department of Biology in the Faculty of Mathematics and Natural Sciences (I) of the Humboldt-Universität zu Berlin is announcing a

W3 Professorship for Neuronal Plasticity

to be filled at the nearest opportunity.

The Professorship in Neuronal Plasticity should analyse the convertibility of neuronal processes including synaptic transmission in the nervous system from a broad neurobiological and pathophysiological perspective. The professorship will work in close cooperation with 15 other new NeuroCure professorships as well as the Cluster's more than 25 existing workgroups. The new NeuroCure building will be located on the Charité Mitte Campus in Berlin in direct proximity to the Department of Biology, the DRFZ and the MPI for Infection Biology. The location thus offers numerous cooperation possibilities with workgroups, collaborative research centers (SFBs), research training groups (Graduiertenkollegs) and other joint research projects supported by these institutions. The professorship should contribute to the foundational training in the bachelor of biology degree program, in the master and PhD degree programs in molecular and organismic biology, biophysics, medical neurosciences and computational neuroscience and in the research training group Mind & Brain. During the funding period, the professorship is responsible for a four-hour teaching load.

The candidate must fulfill the requirements for appointment as professor as set forth in § 100 of the Berlin University Law (Berliner Hochschulgesetz).

As the Humboldt-Universität zu Berlin strives to increase the proportion of women in science and teaching, female candidates are particularly encouraged to apply. Applications from abroad are welcome. In the event of equal personal aptitudes and qualifications, disabled persons will be given priority.

Please address your application within 4 weeks, under the reference number PR/025/09, to the Humboldt-Universität zu Berlin, Dean of the Faculty of Mathematics and Science I Prof. Dr. Schön, Unter den Linden 6, 10099 Berlin, Germany. Since we cannot accept email applications, please send your printed application to the address given above.

If you have any further questions, please do not hesitate to contact us.

NeuroCure Office: Tel: +49 (0)30 450 539 702, neurocure@charite.de, www.neurocure.de



Institute for Structural and Molecular Biology (ISMB)

At University College London – Birkbeck College London

Lectureship in "Biophysical Mass Spectrometry"

The Institute for Structural and Molecular Biology (ISMB) is seeking applications to fill a lectureship in Biophysical Mass Spectrometry.

We want to recruit an outstanding individual who is capable of developing world-class research in the development and application of mass spectrometry (MS) techniques and strategies in biophysics (for example the identification and characterisation of protein interactions, the characterisation of higher order protein structure and/or the characterisation of noncovalent complexes). Successful applicants will also be expected to make an appropriate contribution to the affiliated departments portfolios of graduate and undergraduate teaching.

The salary range offered for this post will be within the University Lecturer (G8: £39,313 - £46,403 per annum inc. LW) range according to experience. The job description and person specification can be found at www.ismb.lon.ac.uk/JD1MS.html and at the respective departmental web sites (<http://www.smb.ucl.ac.uk/> and <http://www.cryst.bbk.ac.uk/>).

To apply, please visit the UCL Vacancies website and follow the on-line application process:

<http://www.ucl.ac.uk/hr/vacancies/adverts/job-list.html>. CVs should contain an account of applicant's current research activities together with a plan of future research intentions. The Head of the ISMB, the UCL Research Department of Structural and Molecular Biology and of the Birkbeck Department of Biological Sciences is Professor Gabriel Waksman. He can be contacted informally for general information (email g.waksman@ucl.ac.uk or g.waksman@bbk.ac.uk).

Deadline for applications is 30th November 2009.

UCL Taking Action For Equality.



The University of Texas at Austin

Endowed Chair Position The Institute for Cellular and Molecular Biology

The Institute for Cellular and Molecular Biology invites applications for an Endowed Chair Position. An academic appointment at the level of tenured Professor will be held in an appropriate academic unit in the College of Natural Sciences. Candidates should have an outstanding research program that applies molecular biological and/or biochemical approaches to important biological problems. Areas of interest include, but are not limited to, Biochemistry, Cancer Biology, and Human Genetics. The position carries an exceptional salary and start-up package.

Building on a strong existing faculty, the Institute has recruited more than 60 new faculty members in the past ten years. Faculty roster can be reviewed at <http://www.icmb.utexas.edu>. In addition to its highly interactive and interdisciplinary research environment, The Institute is the home base for the University-wide Graduate Program in Cell and Molecular Biology and supports the state-of-the-art core facilities for DNA and protein analysis, mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, X-ray crystallography, mouse genetic engineering, and NexGen genomic sequencing. An MD-PhD program with the UT Medical Branch and the new Dell Pediatrics Research Institute in Austin further enhance the environment for Biomedical Research.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send curriculum vitae, summary of research interests, and names of five references to:

Dr. Alan M. Lambowitz, Director
The Institute for Cellular and Molecular Biology
The University of Texas at Austin
1 University Station A4800
Austin TX 78712-0159

The University of Texas at Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply; a background check will be conducted on applicant selected.



Ion Channel Research Unit Duke University Medical Center

Tenure Track Faculty Position Assistant Professor/Associate Professor

The Ion Channel Research Unit (ICRU) at Duke University Medical Center invites applications for a tenure track faculty position.

The candidate should be a scientist or a physician-scientist with a laboratory research program in an area of membrane excitability and/or ion channel structure/function and/or ion channel physiology. Among the broad research areas relevant to this search are programs focusing on channelopathies, neuropsychiatric disorders and neuronal function, peripheral nociception, optogenetics, structural biology, and development.

The ICRU is a multi-departmental and interdisciplinary group of investigators organized around membrane excitability. The laboratory of the successful applicant will be within ICRU space adjacent to ICRU members and the applicant will receive a primary appointment in an appropriate basic or clinical department within the medical school. Opportunities to interact with and build upon programs relevant to membrane excitability include those in neuroscience, cardiac electrophysiology, structural biology, hormone signaling, renal physiology, and development.

The candidate is expected to work primarily as laboratory researcher. High priority will be placed on creativity in laboratory research, potential for independent funding, and dedication to excellence in teaching. A generous start up package commensurate with the candidate's academic level will be provided.

Interested individuals should submit a CV, statement of research interest, and request that three letters of references to be sent as PDF files directly to: icru@mc.duke.edu. The deadline for receipt of applications is **December 8, 2009**.

*Duke University Medical Center is an
Equal Opportunity/Affirmative Action Employer.*



Assistant or Associate Professor MODELING NEUROBIOLOGICAL SYSTEMS/COMPUTATIONAL NEUROSCIENCE

The Division of Biological Sciences (www.biology.missouri.edu) at the University of Missouri-Columbia invites applications for a tenured or tenure-track position in modeling neurobiological systems / computational neuroscience. **We are particularly interested in candidates who develop, expand and evaluate modeling tools to study neurobiological problems.** This is the second in a series of hires designed to increase computational/modeling approaches in our department. The successful candidate will join a diverse group of biologists with interests in neurobiology and behavior, cell and developmental biology, and ecology and evolutionary biology. There are strong possibilities for joint appointments with either Biological Engineering or Electrical/Computer Engineering if the candidate and department(s) are interested.

We offer a highly competitive salary and start-up package, an active doctoral program with institutional and federal support for students, and a highly interactive faculty.

Send application by e-mail to: neuromod@missouri.edu. Attach a single Adobe Acrobat PDF or Microsoft Word document that includes your vita and statements of research and teaching interests. Have three letters of reference mailed to: **John David, Chair, Division of Biological Sciences, 105 Tucker Hall, University of Missouri, Columbia, MO 65211-7400**. Review of applications will begin **December 15, 2009**. To request ADA accommodation contact **Johnette Blair** at **573-882-6650** or BlairJo@missouri.edu.

*MU is an Equal Opportunity-Affirmative Action Employer:
We are committed to ethnic, racial, and gender diversity in our faculty and strongly encourage applications from women and members of groups underrepresented in science.*

POSITIONS OPEN

ASSISTANT PROFESSOR Department of Physiology and Biophysics Dalhousie University

The Department of Physiology and Biophysics invites applications for a position at the rank of Assistant Professor for an individual with research interests in the electrophysiology of excitable cells and tissues. Candidates whose research incorporates modern molecular biology and imaging techniques will be given preference. Applicants must have a Ph.D., M.D., or equivalent degree, postdoctoral training, excellent communication skills, and a strong record of peer-reviewed publications. The successful candidate will be expected to develop active and synergistic research collaborations with other basic science or clinical researchers in the Faculty of Medicine, to develop an extramurally funded research program, and to participate in the teaching mission of the Department. This is a salaried, probationary, tenure-track appointment; however, the candidate will be encouraged to apply for external salary support from appropriate granting agencies.

Interested applicants should submit curriculum vitae along with a brief description of research and teaching experience and interests, and arrange to have three letters of reference sent directly to: **Dr. Paul R. Murphy, Head, Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University, Sir Charles Tupper Medical Building, 5850 College Street, Halifax, Nova Scotia B3H 1X5 Canada.**

Review of applications will begin in November 2009 and will continue until the position is filled.

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. Dalhousie University is an Employment Equity/Affirmative Action Employer. The University encourages applications from qualified Aboriginal people, persons with a disability, racially visible persons, and women.

POSITIONS OPEN



Texas A&M University invites qualified applicants to apply for the position of **PROFESSOR AND HEAD**, Department of Entomology in College Station, Texas. Applications will be accepted through January 15, 2010, or until a suitable candidate is selected. Position is available June 1, 2010. Specific duties and responsibilities include: (1) provide intellectual and philosophical leadership of faculty, staff, and students for integrated academic, research, and extension programs, and with expanding international activities; (2) manage and coordinate departmental human, fiscal, and physical resources; (3) serve as primary advocate for the Department to the administration in the College of Agriculture and Life Sciences and The Texas A&M University System agencies, and lead in the development of innovative joint ventures with other academic units; (4) provide leadership for statewide entomological programs of Texas; (5) represent the Department to commodity groups, state and federal agencies, other partners and collaborators; and (6) provide leadership for continued acquisition of internal and external resources. See detailed announcement at website: <http://insects.tamu.edu/departments/headsearch/>. Submit inquiries to e-mail: entodhsearch@ag.tamu.edu. Applications should be submitted to website: <http://Greatjobs.tamu.edu> NOV# 04537.

We deliver
customized job alerts.

www.ScienceCareers.org

POSITIONS OPEN

THEORETICAL/QUANTITATIVE EVOLUTIONARY BIOLOGIST

The Department of Biology, University of Florida, seeks a faculty member working at the interface between evolutionary biology and infectious disease, who will be expected to have an adjunct appointment in the UF Emerging Pathogens Institute (EPI). We seek applicants who develop quantitative and theoretical tools to advance our understanding of the evolution of emerging pathogen systems, and may also use such tools to inform broader domains of biological inquiry. Instruction at both undergraduate and graduate levels is expected. For more information and to apply, see website: <http://www.biology.ufl.edu/Administration/Jobs.aspx> (position #00025351). The appointment is at the rank of tenure-track **ASSISTANT PROFESSOR**; however, appointment at the **ASSOCIATE PROFESSOR** rank is possible for an exceptional candidate. Ph.D. or equivalent in a relevant discipline is required; postdoctoral research experience is preferred. Salary is commensurate with experience. Review of applications begins 1 December 2009 and will continue until the position is filled. *Women, minorities and members of other underrepresented groups are encouraged to apply. The University of Florida is an Equal Opportunity Institution.*

BIOLOGY DEPARTMENT CHAIR. Indiana University-Purdue University Indianapolis (IUPUI) seeks an outstanding individual with vision, scientific standing, and leadership qualities to chair the Department of Biology. The new chair will lead significant expansion of a Department with existing strengths in research and undergraduate and graduate teaching. Qualifications for the position include a significant record of research, teaching, and leadership appropriate for appointment as **FULL PROFESSOR**. See website: <http://www.biology.iupui.edu/BioChairSearch.pdf> for information and application instructions. IUPUI (website: <http://www.iupui.edu>) is an Equal Opportunity/Affirmative Action Institution.



KECK GRADUATE INSTITUTE
of Applied Life Sciences

Research Faculty Positions in Applied Life Sciences

Applications are sought for research faculty at the level of assistant, associate, or full professor to participate in the development and growth of our unique academic centers: The Center for Biomarker Research, the Center for Rare Disease Therapies, the Center for Network Studies, and the Amgen Bioprocessing Center. We seek entrepreneurial individuals dedicated to excellence in the applied life sciences, with a PhD in a relevant science or engineering field, who currently have research funding and who will be able to support their research through external funding from federal agencies, private foundations, or through industry sponsored translational research and development activities. Startup funds, laboratory space including wet labs for business incubators, and access to shared facilities and research infrastructure will be provided. For further details, please visit <http://www.kgi.edu/x9983.xml>. Keck Graduate Institute, a member of the Claremont Colleges, is small and interdisciplinary, dedicated to education and research, with no departmental boundaries, and with strong connections to the Life Science Industry. Applications should include a cover letter describing interests, plans for research, and desired center affiliation, a CV, a list of recent, current, and pending research support, and a list of 3 contacts for references. For appointments starting Spring or Summer of 2010, submit applications via email by **December 15, 2009** to Research Faculty Search Committee, Keck Graduate Institute of Applied Life Sciences, faculty-search@kgi.edu.

KGI is an Equal Opportunity Employer.



Anthropogenic Climate Change – Princeton University Post Doctoral Research Position: Anthropogenic Climate Change 2009-2010

The Program in Science, Technology, and Environmental Policy at the Woodrow Wilson School of Public and International Affairs at Princeton University invites applications for post-doctoral Research Associates for research on anthropogenic climate change under the direction of **Professor Michael Oppenheimer**. General areas of interest include impacts and vulnerability of human and natural systems, and domestic and international policy frameworks aimed at fostering greenhouse gas emission mitigation and adaptation.

Recent research in this program has focused on: the vulnerability of coral reefs, the nitrogen cycle and its response to climate variability and change, paleoclimatic studies of the behavior of earth's ice sheets and sea level and implications for the future, the impacts of sea level rise on the coastal zone, the human responses to climate impacts including migration, the application of climate modeling to the foregoing problems, energy policy including the appropriate role for bio fuels, the role of uncertainty and learning in decision making on problems of global change, and various aspects of emissions trading. This research is motivated by an interest in climate policy on all time scales and particularly, the means to implement Article 2 of the UNFCCC ("avoiding dangerous anthropogenic interference with the climate system"). Potential applicants are encouraged to explore papers and research projects described at: <http://www.princeton.edu/step/people/faculty/michael-oppenheimer/>.

Applicants should have a strong background in one or more of the areas above and an interest in attacking problems from a multidisciplinary perspective, including the exploration of implications for public policy. Research is often carried out in collaboration with natural and social scientists in the Woodrow Wilson School, as well as Princeton's Department of Geosciences, the Atmosphere and Ocean Sciences Program, the Department of Ecology and Evolutionary Biology, and NOAA's Geophysical Fluid Dynamics Laboratory. The initial appointment will be for one year, with the possibility of renewal. The Postdoctoral Research Associate's position is open to all regardless of citizenship, but requires a completed doctorate in a relevant area of environmental science and does not support work towards the completion of a degree. The research associate position will be eligible for salary and full employee benefits in accordance with Princeton University guidelines.

Applicants should apply at the Princeton University jobsite, <https://jobs.princeton.edu> (use requisition number **0900424**) The search begins immediately and continues until the position is filled. For information about applying to Princeton and voluntarily self-identifying, please link to http://www.princeton.edu/dof/about_us/dof_job_openings/.

Princeton University is an Equal Opportunity Employer and complies with applicable EEO and affirmative action regulations. Princeton University is an Equal Opportunity/Affirmative Action Employer.

FRED HUTCHINSON CANCER RESEARCH CENTER

A LIFE OF SCIENCE

PRESIDENT AND DIRECTOR

Fred Hutchinson Cancer Research Center (FHCRC) is a world leader in research to understand, treat and prevent cancer, HIV/AIDS and other life-threatening diseases. Founding members of the Center are credited with pioneering bone-marrow transplantation as a successful treatment for leukemia and other blood diseases. Today, FHCRC is an institution of world-renowned depth and variety and home of more than 2,800 scientists and staff, including three Nobel laureates.

FHCRC is seeking a dynamic and nationally recognized leader to assume oversight of the Center and its direction as President and Director. This role represents an exceptional opportunity to shape and lead a diverse team of scientists conducting cancer-related research and advancing the transfer of new diagnostic and treatment techniques from the research setting to patient care. The President and Director (President) of FHCRC is responsible for strengthening and advancing the basic, public health sciences, human biology, clinical research, and vaccine and infectious disease research which is FHCRC's mission. The ideal candidate will bring a M.D. or Ph.D. and exceptional management and administrative skills derived from a senior management role within a highly complex and diverse research organization. The ideal candidate will have a scientific background from a broad set of disciplines or notable accomplishments within his/her focused area of expertise. The ideal candidate will be a highly charismatic, energetic, and diplomatic leader with the interpersonal skills and intellect needed to lead FHCRC.

Russell Reynolds Associates is working closely with the Search Committee to facilitate this important search. Please forward letters of interest or recommendation to:

Fred Hutchinson Cancer Research Center
C/O Carol Emmott/Nalini Perkins
Russell Reynolds Associates
1701 Pennsylvania Avenue NW
Suite 400
Washington, DC 20006
mmaloney@russellreynolds.com

GMI GREGOR MENDEL INSTITUTE OF MOLECULAR PLANT BIOLOGY



Group Leader Position (Population Genetics, Genomics and Evolution)

Gregor Mendel Institute, Vienna, Austria

The **Gregor Mendel Institute of Molecular Plant Biology (GMI)**, founded by the Austrian Academy of Sciences in 2000, is a leading institute in the field of plant molecular biology located at the prestigious Vienna Biocenter Campus. Current research topics include population genetics, epigenetics, chromosome biology, developmental biology and plant stress signal transduction. For more information, see website: www.gmi.oew.ac.at

The research topic of the new Group Leader should be in the broad field of plant genomics, systems biology, or population genetics, preferably with a connection to evolution. Side projects involving organisms other than plants are welcome.

The GMI offers internationally competitive packages, including substantial institutional funding and access to state-of-the-art core facilities. The initial contract will be for 5 years (extension is subject to review).

Please send your application, including a curriculum vitae, a description of your proposed research, and contact details for three referees to Christiane Haffner (christiane.haffner@gmi.oew.ac.at). Review of applications will begin on 1 December 2009. Informal inquiries can be directed to Dr. Magnus Nordborg (magnus.nordborg@gmi.oew.ac.at).



ASSISTANT OR ASSOCIATE PROFESSOR
Cognitive Neuroscience (tenure track)
University of Washington Institute for
Learning and Brain Sciences

The University of Washington's Institute for Learning and Brain Sciences (I-LABS), an interdisciplinary brain research center, has a tenure-track faculty opening for an Assistant/Associate Professor in cognitive neuroscience with a focus on language. Departmental affiliation can be in Psychology, Speech and Hearing Sciences, Linguistics, or Biology, depending on the applicant's background and training. Ph.D. required. Appointment at the Associate Professor level will be considered for candidates who have an outstanding research record. I-LABS faculty study life-long learning and specialize in human cognitive development and learning. We have a growing developmental group at the Institute. The Institute will open its own Magnetoencephalography Brain Imaging Center in April 2010. The successful candidate will be one who brings expertise in human cognitive neuroscience with a focus in the domain of language, development, and/or research using MEG/event-related potentials. Faculty responsibilities will begin September 16, 2010.

Applicants should send a statement of teaching and research interests, curriculum vitae, up to five publication reprints, and three letters of recommendation to: **Patricia Kuhl, Co-Director, Institute for Learning and Brain Sciences, Mailstop 357988, University of Washington, Seattle, WA 98195. E-mail: pkuhl@u.washington.edu.** Review of applications will begin in January 15, 2010, and will continue until the position is filled.

The University of Washington is an Affirmative Action, Equal Opportunity Employer, and is building a culturally diverse faculty and staff and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans. UW faculty engage in teaching, research, and service. The University of Washington, a recipient of the 2006 Alfred P. Sloan award for Faculty Career Flexibility, is committed to supporting the work-life balance of its faculty.

ASSISTANT PROFESSOR, MICROBIOLOGIST
Assistant Professor of Biology, Tenure Track
Bridgewater State College
Department of Biological Sciences

In search of a Microbiologist who can support the biomedical concentration, is strongly committed to excellence in undergraduate teaching, in mentoring original undergraduate research, and in advising, including the sharing of advising responsibilities for undergraduates interested in the biomedical and health professions. Teaching requirements will include microbiology, introductory biology, and a first- and/or second-year seminar course. In addition, the applicant will teach an upper-level elective course, or courses, in the area of expertise that will complement and support the biomedical concentration. Candidates with expertise in immunology are of special interest, but microbiologists with expertise in other areas that complement our departmental offerings are also encouraged to apply. The successful candidate must have excellent communication skills and an earned Ph.D. by May 2010. Postdoctoral and teaching experience is preferred. Applicants should be strongly committed to excellence in teaching and advising, and to working in a multicultural environment that fosters diversity. They should also have an ability to use technology effectively in teaching and learning, the ability to work collaboratively, evidence of scholarly activity, and a commitment to public higher education. Salary commensurate with experience. Interested applicants should apply online at **website: <http://jobs.bridgew.edu>** and attach the following documents to their online application: a letter of interest, curriculum vitae, and statements of teaching and research. Review of applications will continue until the position is filled. *Bridgewater State College is an Affirmative Action/Equal Opportunity Employer that actively seeks to increase the diversity of its work force.*



The Department of Oncological Sciences of the Mount Sinai School of Medicine is seeking **ACADEMIC TRACK FACULTY** at all levels. Candidates must have Ph.D., M.D., or dual degrees and a strong background in research relevant to cancer biology.

Applicants should submit curriculum vitae, including contact details for at least three references, and a summary of research accomplishments and plans for future research directions no later than December 29, 2009. Applications may be submitted electronically to **e-mail: oncsd.search@mssm.edu** or by surface mail to: **Search Committee, Department of Oncological Sciences, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1130, New York, NY 10029.**

BIOPHYSICIST

Wilkes University invites applications for a tenure-track **ASSISTANT PROFESSOR** appointment in the Department of Biology and Division of Engineering and Physics.

We seek a candidate with expertise in biophysics, biomechanics, or bioengineering, with the ability to teach and conduct research at the interface of biology and physics in an undergraduate setting. The appointment requires a Ph.D. in a relevant field, and post-doctoral experience is preferred.

The successful candidate will develop a strong research program involving undergraduates and will participate in a project to revise the curricula in physics and biology. Teaching responsibilities will include introductory physics and upper-level undergraduate courses in an area of expertise. A competitive startup package, supported with funding from the Howard Hughes Medical Institute, is available.

Applicants should submit a letter of application along with their curriculum vitae, statements of teaching and research goals, and recent reprints (PDF) to: **Wilkes University, Biophysics Assistant Professor Search, Reference #BIO004, P.O. Box 3924, Scranton, PA 18505-0924.** Indicate the reference number on the envelope. To apply electronically, send application materials to **e-mail: caply@wilkes.edu** and indicate the reference number in the e-mail subject line. Kindly arrange to have three letters of reference sent to the same address. Please include the reference number, or the application will not be processed.

Please submit all materials by November 23, 2009. For further information about this position, please contact the **Chair of the Search Committee, Dr. William Terzaghi, e-mail: william.terzaghi@wilkes.edu.**

Wilkes University is an Equal Opportunity Employer committed to a diverse faculty, staff, and student body. Applicants from diverse backgrounds are strongly encouraged to apply.

Black Hills State University seeks a broadly trained **INORGANIC CHEMIST** for a tenure-track position to start August 2010. Ph.D. in chemistry is required. Primary duties include teaching inorganic chemistry for undergraduates, establishing a research program, and grant writing. Candidates must have research interests in the area of photoactive nanoscale systems or be able to take advantage of the nearby deep underground laboratory. To apply go to **website: <http://yourfuture.sdbor.edu>** and provide a letter of interest, curriculum vitae, and a statement of teaching experience, teaching philosophy, and research interests. Have three letters of reference sent directly to: **Dr. M. Zehfus, Unit 9088, 1200 University Street, Spearfish, SD 57799.** Review of applications will begin November 13, 2009, and continue until position is filled.



The USDA, Agricultural Research Service, Cereal Disease Research Unit in St. Paul, Minnesota, is seeking a **RESEARCH SCIENTIST (MOLECULAR BIOLOGIST/PLANT PATHOLOGIST/GENETICIST)**, GS-12 (Announcement Number ARS-X9W-0266), salary range \$71,473.00 to \$92,911.00. The scientist will conduct research to address a range of research issues associated with rust diseases of wheat and barley and their mitigation. The incumbent will conduct research on host resistance, pathogen biology, and genetics pertaining to the interaction between Ug99 stem rust and its cereal host. The incumbent is expected to identify and characterize interactions between cereal rust pathogens and their hosts using modern tools such as genomics, functional proteomics, or metabolomics. The incumbent is expected to publish research results in peer-reviewed journals and to give research presentations at national and international scientific meetings and conferences. *United States citizenship is required.* For information on the research program and/or position, contact **Pam Groth, telephone: 651-649-5046; e-mail: pam.groth@ars.usda.gov.**

Refer to **website: <http://www.ars.usda.gov/Careers/Careers.htm>** for further information and complete application instructions or call **telephone: 301-504-1583.** Send application materials to: **Latania Maise, USDA-ARS-HRD-WSB, 5601 Sunnyside Avenue, Stop #5106, Beltsville, MD 20705-5106** by November 30, 2009. *USDA/ARS is an Equal Opportunity Provider and Employer.*

ASSISTANT PROFESSOR OF BIOLOGY
Arcadia University

The Department of Biology invites applications for a full-time, tenure-track Assistant Professor position in neurobiology beginning fall 2010. Ranked among the top tier of comprehensive universities in the northern region of the United States, Arcadia University is a coeducational institution located in suburban Philadelphia that offers undergraduate and graduate study to more than 3,500 students annually. The successful candidate will teach an upper-level neurobiology elective to biology majors and in the general biology sequence. Expertise in other areas meeting departmental teaching needs will be considered. Applicants are expected to engage undergraduate students in neurobiology-related research projects, so postdoctoral research experience is desired. We especially encourage applications from candidates able to employ a variety of approaches to one or more of the following subdisciplines of neurobiology: developmental neuroscience, molecular and cellular neuroscience, neuroendocrinology, and systems neuroscience. Candidates must have an earned doctorate, a dedication to teaching biology majors and nonmajors, and teaching experience at the university level. For full consideration, submit letter of intent, statement of teaching philosophy, statement of research goals, curriculum vitae, and three letters of recommendation by December 15, 2009, to: **Dr. Archie Vomachka, Department of Biology, Arcadia University, 450 S. Easton Road, Glenside, PA 19038. Telephone: 215-572-2199; fax: 215-881-8758; e-mail: vomachka@arcadia.edu.** *Arcadia University seeks candidates of diverse cultural backgrounds and abilities. As an Affirmative Action/Equal Opportunity Employer, Arcadia University encourages members of underrepresented groups to apply.*

FACULTY - DEVELOPMENTAL BIOLOGY

The University of New England invites applications for a tenure-track faculty position in developmental biology. Details are available at **website: <http://www.une.edu/hr/ads/asstprofdevbio>.** Applicants should submit electronic copies of their curriculum vitae and statements of teaching philosophy and research interests and should have three letters of recommendation sent to: **Ms. Isabelle Yokana, e-mail: iyokana@une.edu.** Review of applications will begin immediately and continue until position is filled. *UNE is an Equal Opportunity/Affirmative Action Employer and strongly encourages candidates of diverse backgrounds.*



Plant Biology Faculty Positions

The Department of Biology at Penn State University invites applications for tenure-track faculty positions at all ranks in **Plant Biology**, with research programs in molecular, cellular, and/or developmental aspects of plant biology. We particularly encourage applications from individuals whose work is related to energy storage in plants (including carbon partitioning; synthesis of cell walls, starch and lipids; and genetic control of these metabolic pathways) as well as other areas related to the physiology and development of land plants. Penn State is an international center for plant biology research, with particular emphases in cell biology, development, genomics, ecology, and evolution. Penn State has an outstanding interdisciplinary plant biology graduate program, excellent plant growth facilities, and a number of core resources that provide research support, including bioimaging, molecular biology, genomics, and proteomics. Interaction with existing research programs is particularly encouraged. Prospective candidates should have a strong record of research accomplishments, ability to secure extramural grants, and a commitment to teaching. Candidates should email a letter of application, *curriculum vitae*, research prospectus, statement of teaching interests, and names and contact information of at least three references, all as a single PDF document, to plantenergy3@bio.psu.edu. Publications and manuscripts (not more than three) may also be submitted as separate PDF documents along with the application. Review of completed applications will begin **November 23, 2009**. Penn State is committed to Affirmative Action, Equal Opportunity and the diversity of its workforce.



Faculty Positions in Burnett School of Biomedical Sciences College of Medicine

Cancer, Cardiovascular and Metabolic Diseases, Infectious Diseases and Neurodegenerative Diseases

University of Central Florida is expanding its Biomedical Research and Education Program into a new 198,000sq.ft. Burnett Biomedical Science building in the new medical campus. We seek outstanding scientists working on molecular, cellular, physiological, biochemical, or pharmacological approaches to study important problems with relevance to cancer, cardiovascular and metabolic diseases, infectious diseases and neurodegenerative diseases. Faculty at Assistant, Associate or Full Professor level will be considered. Successful applicants will be expected to establish a well funded research program, contribute to teaching, and actively participate in MS and PhD programs.

Competitive salaries, startup funds, new laboratories, transgenic animal facilities and access to shared core instrumentation facilities will be provided. Burnett School researchers will have access to the extensive core facilities in the adjacent Burnham Medical Research Institute. Medical campus is part of multibillion dollar biomedical cluster that will include the new Medical School, Burnham Medical Research Institute, Veterans Administration Hospital and Nemours Children's Hospital.

The University of Central Florida has over 53,000 students and an outstanding technology-based infrastructure. It is located in Orlando, a dynamic and progressive metropolitan region, a major player in high-tech industry, and adjacent to a top ranked Research park and a great place to live and work.

Review of candidates will begin on **November 20, 2009**. Please apply by submitting a curriculum vitae, a two page summary of research plans and contact information for three or more references to biomed@mail.ucf.edu.

The University of Central Florida is an Equal Opportunity, Equal Access, and Affirmative Action Employer. As a member of the Florida State University System, all application materials and selection procedures are available for public review.



Faculty Position in Quantitative Biology University of North Carolina at Chapel Hill

The Department of Biology in the College of Arts and Sciences at the University of North Carolina at Chapel Hill, in conjunction with the Lineberger Comprehensive Cancer Center (LCCC) and the Carolina Center for Genome Sciences (CCGS), seeks a colleague who is applying quantitative and/or computational methods to the study of molecular, cellular or developmental systems relevant to cancer. Examples of appropriate specializations include, but are not limited to, the use of genomics-based, quantitative tools to study physical processes within the cell, developmental processes unfolding at multiple scales, and epigenetic control of genome function. Research may or may not be purely computational. The search is open to any rank, with exceptional junior candidates especially encouraged to apply. The successful candidate will contribute to a new initiative in Quantitative Biology (http://www.bio.unc.edu/News/FacultySearch/UNC_Qbio_Program.pdf) and would likely be affiliated with the NIH-funded graduate Curriculum in Bioinformatics and Computational Biology (<http://bcb.unc.edu>).

TO APPLY: Submit a cover letter ending with up to 5 key words, a CV, a research statement (<3 pages), a teaching statement (<2 pages), and optionally one additional supporting document online at jobs.unc.edu/1002043. Four letters of reference are required, and may be submitted electronically to cpasternak@bio.unc.edu or by mail to: **Quantitative Biology Search Committee, Department of Biology, CB# 3280, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280**. Review of applications will begin **November 16, 2009**, with interviews occurring in February and March 2010. The position will be effective on or after July 1, 2010. The successful candidate must have a Ph.D. in a relevant field by the effective date. The position is open until filled. For inquiries, please contact **Dr. Jeff Dangl** (dangl@email.unc.edu; 919-962-5624) or **Dr. Jason Lieb** (jlieb@bio.unc.edu; 919-843-3228).

The University of North Carolina is an Equal Opportunity Employer.

www.bio.unc.edu

Chair, Department of Biochemistry and Molecular Biology

The University of Arkansas for Medical Sciences (UAMS) College of Medicine invites applications and nominations for the position of Chair, Department of Biochemistry and Molecular Biology. The successful candidate is expected to have a record of outstanding accomplishments in research and education in biochemistry or molecular biology.

The Chair reports to the Dean of the College of Medicine and is responsible for the overall academic, administrative, and fiscal leadership of the department. The department has 15 tenure track faculty with research strengths in many areas, including protein structure/function, glycobiology, signaling, nutrition, membrane biology, and tumor biology. Further information about the department is at <http://www.uams.edu/biochem>.

Review of applications will begin December 1, 2009, and will continue until the position is filled. Email submission is preferred, with curriculum vitae, statement of career goals, and names of three references attached as PDF files. Applications and nominations should be submitted to:

Michael L. Jennings, Ph.D. Chair, Department of Physiology & Biophysics
University of Arkansas for Medical Sciences
4301 West Markham St., #505
Little Rock, AR 72205
Phone: 501-296-1438
Fax: 501-686-8167
Email: JenningsMichaelL@uams.edu



UAMS is an equal opportunity employer and promotes workplace diversity. Women and members of underrepresented minorities are strongly encouraged to apply.

POSITIONS OPEN

FACULTY POSITIONS IN BIOLOGY

Indiana University-Purdue University Indianapolis

The Department of Biology at Indiana University-Purdue University Indianapolis (IUPUI) (<http://www.biology.iupui.edu/>) invites applications for three to five tenure-track faculty positions at any rank, to begin August 1, 2010. We seek candidates with a firm commitment to research excellence who complement and expand departmental and campuswide interdisciplinary research programs in the life and health sciences. Areas of research specialization are open, but we are also interested in addressing undergraduate instructional needs in genetics and molecular biology and in biochemistry. Diverse opportunities for collaboration exist with faculty in the School of Science, the School of Engineering and Technology, and the adjacent Indiana University School of Medicine and its state-of-the-art core facilities, as well as with scientists in nearby pharmaceutical and biotechnology companies. See the Faculty Search file at the biology home page (listed above) for links to IUPUI research resources.

Applicants must have a Ph.D. with postdoctoral experience, must demonstrate evidence of research excellence and the ability to initiate and maintain an externally funded research program, and must have a background appropriate for successful teaching at the graduate and/or undergraduate levels. Applicants at the Associate or Full Professor level must have an established record of research excellence, external funding, and student mentoring. Tenure may be offered to a successful senior candidate, depending on the candidate's qualifications and accomplishments.

Applicants should electronically submit curriculum vitae, a statement of research plans, a description of teaching experience and teaching philosophy, and names and contact information for three references as a single PDF file to Dr. N. Douglas Lees, Chair, Faculty Search Committee, e-mail: biol1fac@iupui.edu. Review of applications will continue until all positions are filled. Application review will commence on 15 November 2009, but those received by 15 December 2009 are assured full consideration.

IUPUI is Indiana's life and health science campus, with over \$300 million in research awards. IUPUI attracts over 30,000 students, offers over 180 undergraduate and 70 graduate and professional degrees, and maintains an attractive urban campus. The School of Science enrolls more than 2,300 majors, including approximately 300 M.S. or Ph.D. students. IUPUI was recognized in the 2009 *U.S. News and World Report* College Rankings as placing seventh of 77 campuses "everyone should be watching" and in the 2008 *Forbes*.com list, Top 15 percent of America's Best Colleges. Indianapolis, the 13th largest city in the United States, is the cultural and political center of Indiana, and was recently ranked as the nation's most affordable major housing market.

IUPUI is an Equal Employment Opportunity/Affirmative Action Employer with a strong commitment to increasing faculty diversity. We seek to attract a diverse applicant pool for this position, including women and members of minority groups.

Georgetown University wishes to recruit a tenure-track ASSISTANT PROFESSOR in physical chemistry to begin fall 2010. Areas of research are open, but proposals related to sustainable energy or environmental chemistry are preferred. Candidates must have a Ph.D. degree in chemistry or a closely related field and significant postdoctoral training or equivalent. Development of an internationally recognized research program and teaching at the undergraduate and graduate levels are expected. Please send curriculum vitae, description of research plans, and statement of teaching philosophy, and arrange for three letters of recommendation to be sent to: Faculty Search Committee, Department of Chemistry, Georgetown University, Box 571227, Washington, DC 20057-1227. For full consideration, complete applications should arrive before November 23, 2009. Georgetown University is an Affirmative Action/Equal Opportunity Employer; applications from qualified women and minority candidates are encouraged.

POSITIONS OPEN

FACULTY POSITIONS

Biochemistry and Molecular Biology
Johns Hopkins Bloomberg School of
Public Health

The Department of Biochemistry and Molecular Biology at the Bloomberg School of Public Health of Johns Hopkins University invites applications for tenure-track faculty positions, preferably at the ASSISTANT PROFESSOR level. We seek outstanding applicants with a demonstrated ability to study problems of major public health relevance at the cellular, molecular, and/or structural level. Areas of particular interest are cancer biology and genome biology. Other favored areas include reproductive biology, molecular nutrition, stress pathways, and aging. Successful applicants will be expected to establish an outstanding research program, be strongly committed to graduate-level training and education, and contribute to the success of the Department and School. Information about the application process and recent faculty hires can be found at [website: http://www.jhsph.edu/bmb/facultysearch](http://www.jhsph.edu/bmb/facultysearch). Applications will be accepted until January 15, 2010. We actively encourage applications from women and minority candidates. The Johns Hopkins University is an Equal Opportunity/Affirmative Action Employer.

MULTIPLE FACULTY POSITIONS IN ENERGY-RELATED RESEARCH University of Notre Dame

As a part of a campuswide sustainable energy initiative, the Colleges of Science and Engineering at the University of Notre Dame invite applications for multiple, open-rank positions, including endowed chairs, in energy-related research. The engineering positions are focused on energy-related separations with areas of interest broadly including the theoretical and experimental development of novel materials and/or approaches for separations using absorption, adsorption, membranes, and biological or hybrid systems. Energy-related applications could include air separation, less energy-intensive groundwater remediation and wastewater treatment methods, water desalination, recovery of dilute organics from aqueous solutions, and actinide/nuclear separations, among others. The science positions are broadly defined to include materials synthesis and characterization, molecular and heterogeneous catalysis, bio- and photochemical energy conversion and storage, as enabled by novel experimental and theoretical chemical science. Successful candidates will bring strong programs in engineering or chemical sciences, will embrace collaborations within and across disciplines, and will incorporate excellence in both undergraduate and graduate education. Primary and joint appointments are envisioned in the Departments of Chemical and Biomolecular Engineering, Civil Engineering and Geological Sciences, Aerospace and Mechanical Engineering, and Chemistry and Biochemistry.

Applicants should submit curriculum vitae, list of publications, detailed research plans, and statement of teaching interests. Junior candidates must also arrange at least three letters of recommendation, while senior applicants can apply in confidence. Applications will be reviewed beginning November 1, 2009, continuing until the positions are filled. Engineering applicants should send materials electronically to e-mail: villarosa.2@nd.edu (subject: Energy Search) or by surface mail to: Energy Search Committee, c/o Barb Villarosa, Notre Dame Energy Center, 182 Fitzpatrick Hall, University of Notre Dame, Notre Dame, IN 46556. Science applicants should use the weblink provided in the Employment Opportunity site at [website: http://chemistry.nd.edu](http://chemistry.nd.edu) or send applications by surface mail to: Energy Search Committee, Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, IN 46556.

The University of Notre Dame, an Equal Opportunity Employer with a strong institutional and academic commitment to diversity, endeavors to foster a vibrant learning community animated by the Catholic intellectual tradition.

POSITIONS OPEN

THE LIBER ERO CHAIR IN FISHERIES RESEARCH Associate or Full Professor Level

The Department of Biology seeks an outstanding individual for a newly created endowed chair dedicated to the research of fish and/or fisheries. The Liber Ero Chair is also supported by an endowment that will generate base research funds. This Chair is being established in the context of a changing climate and ocean, anthropogenic impacts on marine ecosystems, and declining fish populations. The appointee will have an internationally recognized research profile that complements the Department's existing strengths in aquatic sciences. Departmental programs have a strong field component both on and under water, close connections with the School of Earth and Ocean Sciences, and strong collaborations with federal ocean research labs. Applicants expert in all relevant research areas are encouraged, including fisheries oceanography, the role of fish in marine ecosystems, community or population biology of fishes, and fish behavioural ecology or ecophysiology. Applications should address how research will address the response(s) to environmental changes.

The Chair will be occupied by a senior scientist of international renown appointed for a five-year term that is renewable. The appointee is expected to demonstrate research excellence through publication record, funding history, and current projects. In addition, excellent communication skills are essential for education and outreach. The successful candidate will also demonstrate genuine interest in curriculum development at undergraduate and graduate levels. The University of Victoria offers extensive opportunities for research collaborations, including existing fish ecology and genomics programs, the Venus and Neptune Canada cabled observatories, and interactions with researchers at the Bamfield Marine Sciences Centre. Additional research facilities include a coastal research vessel and an extensive aquatics facility. The University's location provides superb access to many marine habitats for research and teaching. More information is available at [website: http://web.uvic.ca/biology/](http://web.uvic.ca/biology/).

The closing date for applications is January 15, 2010. Interested applicants should send curriculum vitae, a teaching dossier, and contact information for three references to:

Dr. William Hintz, Chair
Department Biology
University of Victoria
P.O. Box 3020, Station CSC
Victoria, BC V8W 3N5 Canada

In accordance with the University's Equity Plan and pursuant to Section 42 of the British Columbia Human Rights Code, preference will be given to women, who are encouraged to self-identify. All qualified candidates are encouraged to apply; however, in accordance with Canadian Immigration requirements, Canadians and permanent residents will be given priority.

YALE UNIVERSITY SCHOOL OF MEDICINE Yale Program for Cellular Neuroscience, Neurodegeneration, and Repair Website: <http://info.med.yale.edu/cnnr>

Open position for Director of Advanced Light Microscopy Imaging Facility (part time or full time). Mission of the facility is to make available to members of the Cellular Neuroscience, Neurodegeneration, and Repair program state-of-the-art light microscopy (including two-photon and spinning disk confocal and total internal reflection fluorescence microscopy) and related technical support. The successful candidate is expected to manage the facility, to assist and train investigators, and to keep updated with advancing methodology and equipment.

Ph.D. in physics or biology and experience in microscopy and image processing required. Applications, including cover letter, curriculum vitae, and names of three referees, should be sent electronically to Charlene Bloch, e-mail: charlene.bloch@yale.edu and are due by December 1, 2009.

Applications from, or nominations of, women and minority scientists are encouraged. Yale is an Affirmative Action/Equal Opportunity Employer.

WAYNE STATE UNIVERSITY

Microbiology and Developmental Biology Faculty Positions

The Department of Biological Sciences at Wayne State University has two tenure track openings for new faculty, at either the **ASSISTANT PROFESSOR** or tenured **ASSOCIATE** or **FULL PROFESSOR** levels. We seek individuals studying fundamental problems in microbiology, and in developmental biology. Applicants must have a Ph.D. or equivalent degree, postdoctoral experience, and an established track record of accomplishments appropriate for the level of appointment. A record of outstanding achievement, a promising research program, and a commitment to teaching and service are more important than the particular sub-discipline of these fields of study. We are particularly interested in applicants who use innovative theoretical and/or experimental approaches to advance our mechanistic understanding of living systems. Scientists using multi-disciplinary or integrated approaches are especially encouraged to apply. Applications from dual career couples are encouraged. Applicants are expected to develop and maintain a vigorous, externally funded research program, to participate in graduate and undergraduate education and training, and to participate in service to the department, college, and university. Wayne State University is among the top 50 public research universities as ranked by the National Science Foundation and is noted among the 15 best research institutions at which to work in academia in the nation according to a 2006 survey by The Scientist.

Both positions will officially be posted on-line at jobs.wayne.edu by the end of October. Only those application materials that are submitted to this site will be considered. In addition to an online application that includes cover letter and curriculum vitae, applicants must submit a 2-page statement of their future research plans and have three letters of reference sent to: **Chair, Faculty Search Committee, Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202**. Review of applications will begin **November 16, 2009** and the search will remain open until the positions have been filled. Applications will be considered only when all the materials have been received.

Wayne State University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are especially encouraged to apply.

WAYNE STATE UNIVERSITY

Animal Physiologist Faculty Position

The Department of Biological Sciences at Wayne State University has an opening for a new tenure-track faculty member at the **ASSISTANT PROFESSOR** level. We seek an individual studying fundamental problems in animal physiology. Applicants must have a Ph.D. or equivalent degree, postdoctoral experience, and an established track record of accomplishments. A record of outstanding achievement, a promising research program, and a commitment to teaching and service are more important than the research sub-discipline of physiology. We are particularly interested in applicants who use innovative theoretical and/or experimental approaches to advance our mechanistic understanding of living systems. Scientists using multi-disciplinary or integrated approaches are especially encouraged to apply. The successful applicant will be expected to teach an undergraduate course in human physiology and one other graduate or undergraduate course each academic year. Applicants are also expected to develop and maintain a vigorous, externally funded research program, and to participate in service to the department, college, and university.

Wayne State University is among the top 50 public research universities as ranked by the National Science Foundation and is noted among the 15 best research institutions at which to work in academia in the nation according to a 2006 survey by The Scientist.

The position will officially be posted on-line at jobs.wayne.edu by the end of October. Only those application materials that are submitted to this site will be considered. In addition to an online application that includes cover letter and curriculum vitae, applicants must submit a 2-page statement of their future research plans and have three letters of reference sent to: **Chair, Faculty Search Committee, Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202**. Review of applications will begin **November 16, 2009** and the search will remain open until the position has been filled. Applications will be considered only when all the materials have been received.

Wayne State University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are especially encouraged to apply.



FACULTY POSITION IN BIOLOGICAL CHEMISTRY

The University of New Mexico Department of Chemistry and Chemical Biology seeks to fill a faculty position at the level of Assistant or Associate Professor for Fall 2010. This is a probationary appointment leading to a tenure decision. Candidates are sought in the field of experimental biological chemistry with an emphasis on nucleic acid or protein structure and function, molecular systems biology, or synthetic biology. Minimum qualifications include a PhD in chemistry or a related field. Preferred qualifications at the assistant professor level include a PhD in chemistry, biochemistry or biophysics, post-doctoral experience, and outstanding potential for research and teaching. Preferred qualifications at the associate professor level include the above plus outstanding established research and teaching records. The successful applicant will be expected to demonstrate, as part of the interview process, their ability to teach undergraduate and graduate biological chemistry courses and their ability to build a nationally recognized and externally funded research program.

For best consideration, applicants should apply by **January 15, 2010**. The position will remain open until filled. To apply and to learn more about the position visit our website at http://chemistry.unm.edu/faculty_jobs.php.

The University of New Mexico is an Equal Opportunity Employer.



FACULTY POSITION IN CHEMICAL NANOSCIENCE

The University of New Mexico Department of Chemistry and Chemical Biology seeks to fill a faculty position (tenure-track) at the level of Assistant or Associate Professor for Fall 2010. This is a probationary appointment leading to a tenure decision. Candidates are sought in the field of nanoscience with an emphasis on energy-related research. Minimum qualifications include a PhD in chemistry or a related field. Preferred qualifications at the assistant professor level include a PhD in chemistry, post-doctoral experience, and outstanding potential for research and teaching. Preferred qualifications at the associate professor level include the above plus outstanding established research and teaching records. The successful applicant will be expected to demonstrate, as part of the interview process, their ability to teach undergraduate and graduate chemistry courses and their ability to build a nationally recognized and externally funded research program.

For best consideration, applicants should apply by **January 15, 2010**. The position will remain open until filled. To apply and to learn more about the position, visit our website at http://chemistry.unm.edu/faculty_jobs.php.

The University of New Mexico is an Equal Opportunity Employer.

ANNOUNCEMENTS



AfricaRice

WARDA becomes Africa Rice Center (AfricaRice)

Africa Rice Center, AfricaRice for short, is the new official name adopted for the West Africa Rice Development Association (WARDA) by the Council of Ministers from its member states.

The new name represents the Center's pan-African character as its membership now covers all regions of Africa. Africa Rice Center is an intergovernmental association of African countries. It is also one of the 15 international agricultural research centers supported by the Consultative Group on International Agricultural Research (CGIAR).

POSITIONS OPEN



POSTDOCTORAL/RESEARCH ASSOCIATE POSITIONS are available in the area of mechanism-based cancer prevention using plant-derived compounds and molecular mechanism of metal carcinogenesis in the laboratory of **Xianglin Shi**, Graduate Center for Toxicology, University of Kentucky. Send curriculum vitae to e-mail: xianglin.shi@uky.edu.

POSTDOCTORAL POSITION

Genetic diversity and virulence in *Campylobacter jejuni*
National Food Safety and Toxicology Center,
Michigan State University

We seek to identify outstanding candidates for a Postdoctoral position in the National Food Safety and Toxicology Building at Michigan State University (MSU). The project is focused on the role of genetic diversity in virulence of *Campylobacter jejuni*. The successful candidate will act as project leader and direct the activity of an M.S.-level candidate in the Microbiology program at MSU. The successful candidate should be a very motivated and energetic individual with a strong record of independent research at the Ph.D. level; this person should also be capable of leading a small team of investigators, writing and editing manuscripts and grants, and contributing effectively to a positive research environment. The position is currently funded for at least two years and carries a competitive salary and a generous benefits program.

Candidates should have obtained a recent Ph.D. in microbiology, microbial genetics, or related field (required) with a working knowledge of *Campylobacter* genetics and physiology (preferred). Applications will be accepted until November 30, 2009, or until a suitable candidate is identified.

Qualified applicants should submit curriculum vitae, three letters of reference, and relevant reprints to: **Dr. John Linz**, Professor, Departments of Microbiology and Molecular Genetics, Food Science and Human Nutrition, 234B Trout FSHN Building, Michigan State University, East Lansing, MI 48824-1224. Telephone: 517-355-8474 ext. 126; fax: 517-353-8963; e-mail: jliz@msu.edu.

MSU is an Affirmative Action, Equal Opportunity Institution. Women and minorities are encouraged to apply.

POSTDOCTORAL OPENINGS in Single-molecule Science and Nanobiotechnology

Two immediate NIH-funded Postdoctoral positions in experimental single-molecule biophysics and bionanoscience are available in the group of **Professor Liviu Movileanu** at Syracuse University, Syracuse, New York. The candidate should be a recent Ph.D. in physics, biophysics, chemical biology, or a related field and with experience in at least one of the following areas: single-molecule biophysics, biomolecular engineering, nanofabrication, and ultrasensitive electrical measurements. The successful candidates will employ nanopore probe techniques to detect proteins and nucleic acids by single-molecule stochastic sensing. The initial appointment is for two years with the possibility for renewal positions in the following years. These positions involve both fundamental and applicative research as well as advising graduate students in a highly interdisciplinary and self-contained research team. Salary is negotiable based upon experience and research abilities. Review of materials will begin immediately and applications will be considered until the positions are filled. Qualified candidates should send their curriculum vitae, a list of publications, a short description of their qualifications, and the e-mail addresses/phone numbers of three references to **Professor Liviu Movileanu**, e-mail: lmovilea@physics.syr.edu. Electronic application materials are preferred (please include them as an e-mail attachment).

Further information about the research pursued by Professor Movileanu's group is available at [website: http://www.physics.syr.edu/~lmovilea](http://www.physics.syr.edu/~lmovilea).

POSITIONS OPEN



POSTDOCTORAL FELLOWSHIPS Harvard Medical School

Two NIH-funded Postdoctoral positions are immediately available at Harvard Medical School and Immune Disease Institute. The fellows will study the mechanisms of integrin activation in the context of lymphocyte-endothelial and cancer-stromal cell interactions. We are particularly interested in candidates with prior experience in microRNAs, signaling, and/or biochemistry.

Relevant publications: Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. *Science* 319:627, 2008; The volatile anesthetic isoflurane perturbs conformational activation of integrin LFA-1 by binding to the allosteric regulatory cavity. *FASEB J.* 22:4109, 2008; Aberrant activation of integrin $\alpha 4 \beta 7$ suppresses lymphocyte migration to the gut. *J Clin Invest.* 117: 2526, 2007.

Applications should be sent to:

Motomu Shimaoka, M.D., Ph.D.

Associate Professor

Immune Disease Institute

Harvard Medical School

E-mail: shimaoka@idi.harvard.edu

Website: <http://www.idi.harvard.edu/>

NATIONAL INSTITUTE ON AGING National Institutes of Health

Department of Health and Human Services

The National Institute on Aging (NIA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting for a **POSTDOCTORAL POSITION** in the Laboratory of Cellular and Molecular Biology (LCMB), DNA Repair Unit. Research in the unit focuses on DNA repair mechanisms and oxidative stress and how these processes contribute to age-related diseases.

Candidates must have training in cellular and molecular biology, biochemistry, or a related field and an interest in DNA repair and/or signal transduction. Candidates with experience in DNA repair techniques are desirable. Candidates must have a Ph.D. or M.D. degree and less than five years of postdoctoral experience.

The position is located in Baltimore, Maryland, a city with a stimulating scientific and cultural environment, with proximity to the Johns Hopkins medical campus. Salary commensurate with research experience and accomplishments ([website: http://www.training.nih.gov/stipends.asp](http://www.training.nih.gov/stipends.asp)). Additional information regarding the NIA/IRP and the LCMB is available at the following websites: <http://www.grc.nia.nih.gov>; <http://www.grc.nia.nih.gov/branches/lbc/lbc.htm>.

To apply, send curriculum vitae, a brief description of research interests, and references to: **Michele K. Evans, M.D., c/o Mrs. Virginia Padilla**, National Institute on Aging, National Institutes of Health, 251 Bayview Boulevard, Suite 100, Room 04C221, Baltimore, MD 21224-6825. Or fax: 410-558-8110; or e-mail: padillav@grc.nia.nih.gov.

DHHS and NIH are Equal Opportunity Employers. The NIH is dedicated to building a diverse community in its training and employment programs.

POSTDOCTORAL POSITIONS available at the University of Rochester to investigate molecular mechanisms in acute myeloid leukemia and myelodysplastic syndrome using the mouse model system. Our laboratory focuses on the zinc finger oncoprotein EVI1 and has developed genetic models to investigate its function. Send curriculum vitae and names of three references to **Archibald S. Perkins, M.D., Ph.D.**, e-mail: archibald_perkins@URMC.rochester.edu.

POSITIONS OPEN

NATIONAL INSTITUTE ON AGING

National Institutes of Health

Department of Health and Human Services

The National Institute on Aging (NIA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting for a **POSTDOCTORAL POSITION** in the Laboratory of Cellular and Molecular Biology (LCMB), DNA Repair Unit. Research in the unit focuses on DNA repair mechanisms and oxidative stress and how these processes contribute to age-related diseases.

Ideal candidates must have experience with the single cell gel electrophoresis assay (Comet) and other DNA repair techniques to assess human DNA repair capacity. Candidates must have a Ph.D. or M.D. degree in molecular biology, biochemistry, or a related field, and less than five years of postdoctoral experience.

The position is located in Baltimore, Maryland, a city with a stimulating scientific and cultural environment, with proximity to the Johns Hopkins medical campus. Salary commensurate with research experience and accomplishments ([website: http://www.training.nih.gov/stipends.asp](http://www.training.nih.gov/stipends.asp)). Additional information regarding the NIA/IRP and the LCMB are available at the following websites: <http://www.grc.nia.nih.gov>; <http://www.grc.nia.nih.gov/branches/lbc/lbc.htm>.

To apply, send curriculum vitae, a brief description of research interests, and references to: **Michele K. Evans, M.D., c/o Mrs. Virginia Padilla**, National Institute on Aging, National Institutes of Health, 251 Bayview Boulevard, Suite 100, Room 04C221, Baltimore, MD 21224-6825. Or fax: 410-558-8110; or e-mail: padillav@grc.nia.nih.gov.

DHHS and NIH are Equal Opportunity Employers. The NIH is dedicated to building a diverse community in its training and employment programs.

Multiple **POSTDOCTORAL POSITIONS** available in the Pharmacology/Toxicology Department at the University of Kansas Medical Center to study autophagy, cell death, lipid metabolism, and DNA methylation in the laboratories of **W.X. Ding** ([website: http://www.kumc.edu/pharmacology/Ding.html](http://www.kumc.edu/pharmacology/Ding.html)) and **B.T. Zhu** ([website: http://www.kumc.edu/pharmacology/BaoZhu.html](http://www.kumc.edu/pharmacology/BaoZhu.html)). Interested applicants with training in molecular biology, cell imaging, and animal experimentation should submit their curriculum vitae and names of three references to [website: http://jobs.kumc.edu](http://jobs.kumc.edu) (search for positions J0084743 and J0084953). The University of Kansas Medical Center is proud to be an Equal Opportunity/Affirmative Action Employer.

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Put yourself in the picture by entering the **2010 AAAS Student Poster Competition**. Winners receive a cash prize, framed award certificate, and a one-year AAAS membership—including a subscription to *Science*!



CALL FOR ENTRIES

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American Association for the Advancement of Science

Annual Meeting

18 – 22 February 2010 San Diego

“Bridging Science and Society”

Submission Deadline: Sunday, 1 November 2009

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- All submissions will be peer reviewed. Accepted posters will be listed in the *2010 Annual Meeting Poster Guide*.
- Winners will be published in *Science* and at www.aaas.org/meetings.
- The competition is open to college undergraduate and graduate students only.

To learn more, including how you can volunteer and attend the Annual Meeting for free, visit

www.aaas.org/meetings



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